U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB80-125107

The Bioenvironmental Impact of a Coal-Fired Power Plant, Fourth Interim Report, Colstrip, Montana, December 1978

(U.S.) Corvallis Environmental Research Lab., OR

Apr 79

United States
Environmental Protection
Agency

Environmental Research
Laboratory
Corvallis OR 97330

EPA-600/3-79-044
April 1979

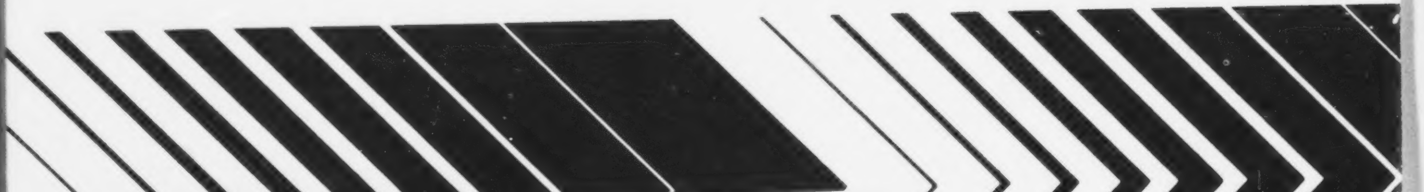
PB80-125107

Research and Development

EPA

Bioenvironmental Impact of a Coal-Fired Power Plant

Fourth Interim Report, Colstrip, Montana December 1978



REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into nine series. These nine broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The nine series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies
6. Scientific and Technical Assessment Reports (STAR)
7. Interagency Energy-Environment Research and Development
8. "Special" Reports
9. Miscellaneous Reports

This report has been assigned to the ECOLOGICAL RESEARCH series. This series describes research on the effects of pollution on humans, plant and animal species, and materials. Problems are assessed for their long- and short-term influences. Investigations include formation, transport, and pathway studies to determine the fate of pollutants and their effects. This work provides the technical basis for setting standards to minimize undesirable changes in living organisms in the aquatic, terrestrial, and atmospheric environments.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/3-79-044	2.	3. RECIPIENT'S ACCESSION NO. PB80-125107
4. TITLE AND SUBTITLE The Bioenvironmental Impact of a Coal-fired Power Plant; Fourth Interim Report, Colstrip, Montana December 1978.		5. REPORT DATE April 1979 issuing date
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Edited by Eric M. Preston and Thomas L. Gullett		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. EPA Corvallis Environmental Research Laboratory 200 S.W. 35th St. Corvallis, OR 97330		10. PROGRAM ELEMENT NO.
		11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS SAME		13. TYPE OF REPORT AND PERIOD COVERED Interim 12/75--12/77
		14. SPONSORING AGENCY CODE EPA/600/02

15. SUPPLEMENTARY NOTES In this series, the 1st Interim Rept. is EPA number EPA-600/3-76-002; the 2nd Interim Rept. is EPA number EPA-600/3-76-013, and the 3rd Interim Rept. is EPA number EPA-600/3-78-021.

16. ABSTRACT

The EPA has recognized the need for a rational approach to the incorporation of ecological impact information into power facility siting decisions in the northern great plains. Research funded by the Colstrip, Coal-fired Power Plant Project is a first attempt to generate methods to predict the bioenvironmental effects of air pollution before damage is sustained. Pre-construction documentation of the environmental characteristics of the grassland ecosystem in the vicinity of Colstrip, Montana began in the summer of 1974. Since then, key characteristics of the ecosystem have been monitored regularly to detect possible pollution impacts upon plant and animal community structure.

In the summer of 1975, field stressing experiments were begun to provide the data necessary to develop dose-response models for SO_2 stress on a grassland ecosystem. These experiments involve continuous stressing of one acre grassland plots with measured doses of SO_2 during the growing season (usually April through October).

Results of the 1976 & 1977 field seasons' investigations are summarized in this publication. The six-year project will terminate in 1980 and a final report will be published after data analyses are complete.

7. KEY WORDS AND DOCUMENT ANALYSIS

DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
plant and animal response to pollution coal-fired power plant air pollutants grassland ecosystems mathematical modeling remote sensing micrometeorological investigation	coal-fired power plant emissions air quality monitoring aerosol characterization	51

DISTRIBUTION STATEMENT release to public	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES 955
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

PA Form 2220-1 (9-73)

★ U. S. GOVERNMENT PRINTING OFFICE: 1979—699-222 /205 REGION 10

EPA-600/3-79-044
April 1979

THE BIOENVIRONMENTAL IMPACT OF A COAL-FIRED POWER PLANT

Fourth Interim Report, Colstrip, Montana
December, 1978

Edited by

Eric M. Preston and Thomas L. Gullett
Terrestrial Systems Division
Corvallis Environmental Research Laboratory
Corvallis, OR 97330

CORVALLIS ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CORVALLIS, OR 97330

DISCLAIMER

This report has been reviewed by the Corvallis Environmental Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

FOREWORD

Effective regulatory and enforcement actions by the Environmental Protection Agency would be virtually impossible without sound scientific data on pollutants and their impact on environmental stability and human health. Responsibility for building this data base has been assigned to EPA's Office of Research and Development and its 15 major field installations, one of which is the Corvallis Environmental Research Laboratory (CERL).

The primary mission of the Corvallis Laboratory is research on the effects of environmental pollutants on terrestrial, freshwater, and marine ecosystems; the behavior, effects and control of pollutants in lake systems; and the development of predictive models on the movement of pollutants in the biosphere.

The Colstrip, Coal-fired Power Plant Project is a first attempt to generate methods to predict the bioenvironmental effects of air pollution before damage is sustained. The results will aid planners in assessing the ecological impact of energy conversion activities on grasslands prior to site selection.

J. C. McCarty
Acting Director, CERL

PREFACE

The Environmental Protection Agency has recognized the need for a rational approach to the incorporation of ecological impact information into power facility siting and managment decisions in the northern great plains. Two capabilities need to be developed. First, in evaluating alternative sites, planners need to be able to predict the kinds and magnitudes of impacts to be expected so that adverse consequences to the environment can be minimized. Secondly, for routine facility management after siting, environmental monitoring methods are needed for detecting incipient ecological damage in time for mitigation efforts to be effective.

Research funded by the Colstrip, coal-fired power plant project is a first attempt to generate the methods needed to predict the bioenvironmental effects of air emissions from coal-fired power plants before damage is sustained. The work can be subdivided into three chronological phases: 1) the identification of information requirements and the expansion of data and information bases to fill these requirements, 2) the integration and synthesis of newly generated data with existing information to define relationships permitting maximum predictive capability, 3) provide the information in a format useful to planners and decision makers involved in siting coal-fired power plants. Several iterations of phases 1 through 3 may be necessary in the long term.

Project rationale and design have been presented in detail in introductory sections of previous interim reports. Until now effort has been largely confined to Phase 1. As the project proceeds, progressively more resources will be devoted to Phases 2 and 3. In the present report, Sections 24 and 25 deal with the synthesis and integration of data bases to generate methods for ecological impact assessment and prediction. The rate of transition in emphasis is primary dictated by the adequacy of data bases available for synthesis.

Research effort for Phase 1 falls roughly into two broad categories: 1) ecological effects monitoring in the vicinity of two 350 megawatt coal-fired power plants at Colstrip, Montana, and 2) field and laboratory process studies designed to elaborate the mechanisms through which coal-fired power plant emissions cause their effects.

Pre-construction documentation of the environmental characteristics of the grassland ecosystem in the vicinity of Colstrip, Montana began in the summer of 1974. This documentation continued until Colstrip generating unit 1 began operation in September, 1975. Since then, key characteristics of the ecosystem have been monitored regularly to detect possible pollution impacts. The current results relevant to this effects monitoring appear in Section 1-8.

In 1974, A Zonal Air Pollution System (ZAPS) was designed to stress 0.5 hectare areas of native grassland with measured concentrations of SO_2. In the summer of 1975, field stressing experiments were begun to provide the data necessary to develop dose-response models of SO_2 stress on a grassland ecosystem. A second Zonal Air Pollution System (ZAPS II), was constructed in 1976. Both ZAPS I and ZAPS II were operated during the growing seasons of 1976 and 1977. The design of these experimental systems has been described in previous interim reports. Their behavior is further described in the present report. Effects on microorganisms, producers and consumers have been monitored throughout the stressing experiments. In addition, field experiments have been conducted to evaluate the effects of SO_2 stress on alfalfa and small grains of agricultural importance in the northern plains. The results of these experiments to date are summarized in Sections 9-20.

Trace element emissions from coal-fired power plants may also influence ecosystem behavior. Laboratory experiments have been conducted to evaluate the behavior of mercury and other selected trace elements on selected soils and plants. These results are presented in Sections 21-23.

The final two sections represent preliminary attempts to utilize information presented in previous sections and previous interim reports to address the project's objectives.

ABSTRACT

The purpose of the Colstrip, Coal-fired Power Plant Project is to develop information that will aid in assessing the potential bioenvironmental impacts of air emissions from coal-fired power plants on northern plains ecosystems before damage occurs. The project has three major components: (1) a case study of the ecological impact of the coal-fired generating units at Colstrip, Montana, (2) a series of field and laboratory process studies designed to elaborate the mechanisms of SO_2 action on grasslands chronically fumigated at low-levels, (3) development of a methodology for incorporating ecological effects information into the power plant siting process.

COLSTRIP STUDIES

Pre-construction documentation of the environmental characteristics of the grassland ecosystem in the vicinity of Colstrip, Montana began in the summer of 1974. This continued until Colstrip generating Unit 1 began operation in September 1975. Since then, key characteristics of the ecosystem have been monitored to detect air pollution and its ecological effects.

Both natural aerosols and those generated by the coal-fired power plants at Colstrip, Montana, were studied. The background aerosols were found to consist of irregular-shaped particles composed primarily of alumino-silicates with relatively high concentrations of the metallic elements for a rural area. Aerosols generated by the power plant were relatively large (approximately 1 μm), glassy, alumino-silicate spheres with a high incidence of calcium and sulfur when collected in the plume near the power plant. At large distances downwind they became small (<.4 μm) sulfur-containing spheres. These artificial aerosols increase the available ice-nuclei concentration by an order of magnitude, and may be significant to the formation and distribution of precipitation.

Plant community studies indicate that graminoids and lichens are the dominant vegetational components influencing cover, number, and diversity at the grassland study sites near Colstrip. The abundance of annual grasses varies markedly among sites, and this strongly affects diversity through reduced equitability. Previous grazing history and variations in yearly climatic conditions, which are primarily responsible for the observed differences in plant species composition, have so far masked any pollution effects. The data suggest a predictive relationship between plant diversity and range condition.

Two lichen species have been monitored for signs of stress since 1974. There were sharp respiration rate increases in September 1977 in both *Usnea hirta* and *Parmelia chlorochroa* at sites closest to Colstrip. Chlorophyll content of *Usnea hirta* thalli may have decreased between 1975 and 1977.

The characteristics of four different years of pine foliage collected during 1977 at pristine and chronically polluted sites are compared. All air pollution damage symptoms are mimicked macroscopically by abiotic and biotic causal agents in pristine environments. The foliar symptoms being measured over time on foliage from chronically polluted areas increased or decreased in comparison to those found in pristine areas.

Pre- and post-operational assessments of the ground level plume contact area were made using terrestrial insects as biological indicators. Post-operational changes in fluoride levels in bee tissues were statistically significant in 1976 and 1977 at downwind sites 15-20 km from Colstrip.

Trends in bird populations have been monitored for three consecutive years in the vicinity of Colstrip. Since 1975, the avian community has become increasingly dominated by Meadowlarks while the relative abundances of raptors, blackbirds and Lark Buntings have decreased. Much of the local variation in bird species diversity is strongly correlated with habitat factors.

Baseline trends in the histology of thymus and thyroid glands of western meadowlarks near Colstrip are reported.

FIELD AND LABORATORY EXPERIMENTS

In 1974, a Zonal Air Pollution System (ZAPS) was designed to stress 0.5 hectare areas of native grassland with measured concentrations of SO_2. Field stressing experiments were initiated during the summer of 1975. A second ZAPS was constructed in 1976. Both ZAPS I and ZAPS II were operated during 1976 and 1977.

Soil and meteorological characteristics at ZAPS are presented.

Average geometric mean SO_2 exposure concentrations for 3 growing seasons (1975, 1976, 1977) on ZAPS I plots were near 1 pphm, 2 pphm, 4 pphm and 7 pphm for the Control, Low, Medium, and High treatments, respectively. Exposure concentrations on ZAPS II for 2 growing seasons (1976, 1977) were near 1 pphm, 3 pphm, 5 pphm, and 7 pphm for Control, Low, Medium, and High treatments, respectively. Standard geometric deviations of SO_2 concentrations on the plots were approximately 3 pphm. SO_2 concentrations on the ZAPS plots showed substantial inter-seasonal and intra-seasonal variation. Concentrations were generally higher at night than during the day, but otherwise patterns of intra-seasonal variability were not consistent between ZAPS plots or between years. Intra-season variation was greatest on High treatment plots and least on Control plots. Seasonal frequencies of SO_2 concentrations were approximately log-normally distributed and showed reasonably good separation in fumigation histories of the treatment plots. Though median SO_2 concentrations of the Control plots were small, these plots were subject to short-term acute fumigations due to drift from other plots. SO_2 concentrations decreased with decreasing height above the ground within the plant canopy.

Soils from each of the fumigation plots were analyzed for their hydrogen oxidation potential using *Alcaligenes paradoxus* as a representative microorganism. After 1 year of fumigation, only the High treatment plot exhibited a detectable depression in hydrogen oxidation potential. However, after 2 years, there was a significant decrease in hydrogen oxidation potential associated with increasing SO_2 exposure.

The dominant grass species on the plots are resistant to acute visible injury from SO_2 exposure. For most, exposure to 1.6 ppm or greater concentrations of SO_2 for four hours or longer is required to produce typical symptoms. Threshold doses for acute injury have apparently not been exceeded even on the highest treatment since no acute visible injury symptoms have appeared.

Chronic visible injury for western wheatgrass, *Agropyron smithii*, on the SO_2 treated plots is expressed by an increased leaf senescence without specific pattern. Leaf senescence occurs earlier and at a more rapid rate than on controls. A gradient of increasing chlorosis from the lower to higher treatment plots was evident in remote imagery after one season of treatment. Unfortunately, within plot heterogeneity made changes of less than 15% in biomass dynamics and net primary productivity impossible to detect with the harvesting method used in 1975. No change attributable to treatment was detected. Harvesting methods with greater precision were used during 1976 and 1977 which allowed changes of about 10% to be detected. To date, no statistically significant changes in net primary productivity have appeared. Fluctuations in species composition and diversity have thusfar shown no consistent relationship to SO_2 treatment.

While the mechanism of SO_2 action has not been demonstrated, field observations and review of the literature allowed development of the following working hypothesis. On the entry to the leaf, SO_2 is rapidly dissolved to form sulfite which is toxic in relatively low concentrations and thought to be responsible for acute leaf injury. Sulfite is slowly oxidized to sulfate which is much less toxic than sulfite and may be used directly as a nutrient. As long as SO_2 is not absorbed at a rate exceeding the cell's capacity to change sulfite to sulfate, acute leaf injury is unlikely. However, as the concentration of sulfate increases over time, toxic levels may be reached causing chlorosis and resulting in an increased rate of leaf senescence.

At the exposure rates on the ZAPS plots, western wheatgrass plants apparently grew at a sufficient rate to incorporate the SO_2 into normal metabolites early in the growing season. Later in the growing season, when metabolic activity slowed, toxic levels of sulfur compounds may have accumulated and accelerated senescence. Thusfar, the increased rate of senescence has been observed only in western wheatgrass on the medium and high treatments. Sulfur accumulation was apparently insufficient to cause early senescence on the low treatments.

Whatever the mechanism, SO_2 causes foliar senescence to begin earlier and therefore reduces the functional life of the leaves of western wheatgrass. This may require that more photosynthate be allocated to maintain active photosynthetic machinery at the expense of other plant processes, such as root storage or seed production. Gradual depletion of root carbohydrate reserves could eventually reduce the capacity of the system to overwinter successfully, sustain grazing, or tolerate additional air pollution.

Sexual reproduction was also apparently impaired by SO_2 treatment on some plots in some species. This was manifested variously as decreases in seed weight, % germination, germination rate, and seed viability depending upon the species. Such effects upon seed production and viability have direct implications for seed farmers in the northern Great Plains, but there may also be significant implications for the viability of the ecosystem. Sexual reproduction provides the genetic mechanism to adapt over the long-term to new environmental stresses such as air pollution. If sexual reproduction is impaired, a smaller range of genotypes will be available upon which adaptive selection can operate.

Grasses on the treated plots appeared to accumulate sulfur in direct proportion to the median SO_2 concentration they experience, though evidence of accumulation on the Control and Low treatments is inconclusive. On the medium and high plots, accumulation proceeds through the growing season. Though much of the accumulated sulfur is cycled through dead above ground material at the end of the growing season, each spring, sulfur accumulation seems to begin from a higher baseline level than was present at the same time during the previous spring. The long-term implications of this are difficult to predict. The increased sulfur availability may prove advantageous to the plants if sulfur levels are deficient and low availability of other nutrients does not limit metabolic utilization of the excess sulfur. If the excess sulfur cannot be utilized, accumulation of toxic concentrations of sulfur compounds may occur progressively earlier in the growing season year after year. This could lead to progressively earlier leaf senescence and accelerate the gradual loss of vitality previously discussed.

SO_2 exposure has apparently modified the forage quality of western wheatgrass plants on the treated plots. Crude protein content decreased from about 10% to about 8% following the second year of exposure on ZAPS I. This change appears to be a very low threshold effect. The reduction in crude protein content occurred even on the low treatment. This effect could have dramatic implications for the stocking capacities of grazing land in the northern Great Plains. Ranchers that can sustain stock by feeding native grasses having 10% crude protein may have to provide a protein supplement if the level were reduced to 8%.

Mycorrhizal fungi are normally associated with the rhizomes of western wheatgrass. This is apparently a mutualistic association in which the fungi utilize a portion of the host's root carbohydrates while facilitating phosphorus transport from humus to the plant. Occurrence of mycorrhizal fungi decreased in direct proportion to increased median SO_2 exposure concentration on the treated plots. The cause of decreased association is still unclear. It is possible that carbohydrate available to the fungi decreased because of decreased photosynthate or increased sulfur translocation to the rhizomes and roots. The significance of decreased mycorrhizal fungal association for the long-term viability of the grasses cannot adequately be evaluated at this time, but any reduction in the ability of primary producers to make use of the otherwise available nutrients would likely be detrimental to primary productivity.

Lichens are an important winter forage for deer and are particularly sensitive to SO_2 stress. Lichen coverage has been severely reduced on the treated plots. Both the amount of photosynthetic tissue and respiration rate are affected.

Though a majority of invertebrate groups failed to show substantial population level treatment responses, there were important exceptions. Ground beetles of the genus *Canthon* consume and/or fragment large quantities of organic material such as feces, carrion, and vegetative matter. Fragmentation creates greater surface area upon which decomposer microflora may work thus facilitating decomposition. These beetles have responded markedly to SO_2 fumigation. Beetle numbers (as measured by pitfall trapping) have decreased dramatically on all treated plots. The exposure threshold causing this effect is apparently quite low. It is not yet known whether reduced numbers result from SO_2 avoidance or a change in life table parameters.

Grasshoppers sever many shoots that they do not consume. This material becomes above ground litter and begins decomposing. This process may reduce total photosynthetic surface of affected plants by removing live shoots and/or it may bring standing dead material into the litter layer where decomposition may occur more rapidly. In any event, the rate of such grasshopper wastage of live and standing dead material must be influenced by grasshopper grazing rates which in turn must be some function of grasshopper numbers and consumption rate per grasshopper. Controlled feeding trials in laboratory cages showed that two grasshopper species selectively rejected western wheatgrass that had been previously exposed to SO_2 on the High treatment plot (ZAPS I). Preliminary work also suggests that grasshopper numbers may decrease on some treated plots. Though the decrease appears to be modest, such effects on grasshoppers could influence the rate of flow of carbon into the litter layer.

Below ground microarthropods, nematodes, tardigrades and rotifers are important to mineralization of nutrients bound in below ground organic material. Any changes in populations of these organisms may substantially affect rates in the below ground portion of nutrient cycles. Responses of below ground organisms to SO_2 treatment has been mixed. Mites have shown no detectable treatment effect. Though total nematode population numbers have not changed, the proportion of saprophagous nematodes has decreased and the proportion of plant feeding nematodes has increased on ZAPS I. Nematodes on ZAPS II have not shown any detectable treatment effects. Tardigrade numbers were reduced in all but one of the treated plots (medium plot, ZAPS II) in 1977. Rotifers appeared to be reduced in the high treatment plots only.

While the magnitude of the above effects on nutrient cycling cannot yet be quantified, several decomposition processes may take longer as a result of SO_2 exposure at levels on the treated plots. A new equilibrium will have to be reached between carbon fixation and nutrient cycling subsystems.

Predatory ground beetles of the family Carabidae have shown a decline in numbers directly proportional to median SO_2 exposure during 1975 and 1976 on both ZAPS sites. This decline in numbers could result from a direct effect of SO_2 on predator avoidance or mortality or an indirect effect due to an effect of SO_2 on prey species. The effects on various arthropod numbers mentioned earlier may affect their availability as food which may in turn affect the population dynamics of small mammals.

A capture-mark-release study of deer mice (*Peromyscus maniculatus*) and prairie voles (*Microtus ochrogaster*) was conducted on the ZAPS sites at monthly intervals from April to September 1976. Throughout most of the trapping period, the number of occupied traps on all fumigated plots decreased relative to Control plots on both ZAPS, and remained relatively higher on Control plots from mid-season to the end of all trapping.

Aerial photography of the ZAPS sites has been collected since 1974. Highly significant correlations were observed between image densities and SO_2 levels on the fumigation plots. Leaf senescence of western wheatgrass showed similar relationships. Color infrared film exposed during the active growth season gave the best results.

Several experiments were conducted at Oregon State University's Schmidt Research Farm to determine the effects of various SO_2 treatments on yield of small grains growing in a field environment. The results of a *chronic exposure experiment* strongly suggested that the yields of Durum wheat and barley could be suppressed by weekly, 72-hour exposures to SO_2 concentrations as low as 15 pphm. Average yield reduction for the 15 pphm treatment were 42% and 44% compared to controls for Durum wheat and barley, respectively. The yield of spring wheat was not reduced by the SO_2 treatment. An analysis of a *multiple exposure experiment* demonstrated that 1) varying the frequency of 3-hour exposures to SO_2 concentrations up to 120 pphm from as often as once every week to as infrequently as once every 5 weeks had no effect on yields of the small grain crops and alfalfa and 2) at 3-hour exposures, increasing SO_2 concentration from 0 to 120 pphm had no effect on yield. A *growth stage experiment* demonstrated there was no growth stage at which the small grain crops or alfalfa were most sensitive to a 3-hour exposure to SO_2 concentrations up to 120 pphm. The growth of tops and roots of range grasses and alfalfa was not affected during the fall season by 3-hour exposure to concentrations of SO_2 up to 120 pphm.

The fate of mercury in five surface soils from southeastern Montana was studied in the laboratory. Western wheatgrass seedlings grown on soils amended with mercuric nitrate showed aerial tissue concentration factors of 0.01 to 0.12.

The uptake of mercury vapor by plants was affected by both illumination and temperature. Among six species examined, the mercury uptake differed between species and a pronounced difference existed between C_3 and C_4 plants, owing to their differences in biochemical processes.

The toxicity of selected trace elements emitted from coal-fired power plants was determined for several biological functions in the blue-green algae, *Anabaena cylindrica*. This organism performs the basic biological

functions of nitrogen fixation, photosynthesis, respiration, and growth, was easily adaptable to existing measurement techniques, is of significance in the grassland biome, and provides an excellent multifunctional organism for testing the effects of emission contaminants. The elements tested were F, Na, Cl, Br, Li, K, Sr, Ba, Cr, Mn, Ni, Cu, Zn, Cd, Hg, Pb, and As. In order of decreasing toxicity Hg, Cu, Cr, Ni, Cd, and Pb exerted strong inhibition at levels of 1 mM or below. In assays of biological functions Hg, Cu, Zn, Cd, and Pb exhibited strong toxicity at 1 mM or lower levels.

CONTENTS

Page

Foreword iii

Preface iv

Abstract vi

List of Contributors xviii

Acknowledgements xxi

Sections

ECOLOGICAL EFFECTS MONITORING IN THE COLSTRIP VICINITY

1. Aerosol Characterization in the Vicinity of Colstrip, Montana
C. C. Van Valin, R. F. Pueschel, D. L. Wellman, N. L. Abshire, G. M. Lerfald, and G. T. McNice 2

Atmospheric Characterization Laboratories 4
Remote Sensing Laboratory 43

2 Baseline Characteristics of Producer and Invertebrate Populations and Certain Abiotic Parameters in the Colstrip Vicinity
J. L. Dodd, J. W. Leetham, T. J. McNary, W. K. Lauenroth, and G. L. Thor 53

Soil and Temporal Dynamics of Soil Water and Precipitation 54
Producers and Primary Production 54
Invertebrate Populations 60

3 Plant Community Monitoring
J. E. Taylor and W. C. Leininger 107

Plant Community Analysis 112
Photographic Studies 119

4 Summary of Observations of *Usnea hirta* and *Parmelia chlorochroa* in the Colstrip Area, 1974-77
S. Eversman 132

5 Foliar Pathologies of Ponderosa Pine Near Colstrip
C. C. Gordon, P. C. Tourangeau, and P. M. Rice 141

Growth/Health/Damage Characteristics of Pine Needles 150
Fluoride, Needle Length, and Fascicular Cross-Sectional Area 171

Anova with GB-1, BNW-1, BNW-2, BNW-3 (1976 and 1977 Collections) 175
Understory Species 186

6 Honeybees and Other Insects as Indicators of Pollution Impact from the Colstrip Power Plants
J. J. Bromenshenk 215

Fluoride 223
Trace Metals 228
Radionuclides 230

7 Trends in Bird Populations in the Vicinity of Colstrip
E. M. Preston and S. K. Thompson 240

Species Composition 242
Seasonal Changes in the Spatial Patterns of Bird Populations 248

8 Baseline Histology of the Thyroid and Thymus Glands of Western Meadowlarks Near Colstrip
M. D. Kern and J. P. Wiggins 257

Normal Histology of the Thyroid Gland of Western Meadowlarks 266
Normal Histology of the Thymus Gland of Western Meadowlarks 270

THE EFFECTS OF CONTROLLED FIELD EXPOSURES TO SO_2 ON BIOLOGICAL SYSTEMS OF THE NORTHERN GREAT PLAINS

9 Temporal Variation in SO_2 Concentration on ZAPS
J. J. Lee, E. M. Preston, and D. B. Weber 284

Interseasonal Trends 293
Intraseasonal Trends 293
Frequency Distributions of SO_2 Concentrations on the Plots 293
Diel Patterns 300
Effect on Micrometeorological Variables on Real Time SO_2 Concentrations 300

10 Spatial Variation of Sulfur Dioxide Concentrations on ZAPS During the 1977 Field Season
E. M. Preston and T. L. Gullett 306

Extensive Study 307
  Vertical Distribution of SO_2 307
  Horizontal SO_2 Distribution 315
  Conversion Factors for Sulfation Values 322
Intensive Study 326

11 Soil and Meteorological Characteristics at ZAPS
J. L. Dodd, W. K. Lauenroth, R. G. Woodmansee, G. L. Thor, and J. D. Chilgren 331

12 The Effect of SO_2 on Soil Microorganism Activity
J. C. McFarlane, R. D. Rogers, and D. V. Bradley, Jr. 377

13 Effects of Chronic Low Level SO_2 Exposure on Producers and Litter Dynamics
J. L. Dodd, W. K. Lauenroth, G. L. Thor, and M. B. Coughenour 384

- Aboveground Plant Biomass Dynamics and Net Primary Productivity 384
- Belowground Plant Biomass Dynamics 388
- Phenology 391
- Effects of Controlled Levels of SO_2 on the Nutrient Quality of Western Wheatgrass and Prairie June Grass 396
    - Sulfur 397
    - Ash 401
    - Crude Protein 401
    - Cell Wall Constituents 404
    - Dry Matter Digestion 404
- Effects of Controlled Levels of SO_2 on Growth and Senescence of Western Wheatgrass 408
- Effects of Controlled Levels SO_2 on Physiology of Western Wheatgrass 428
- Distribution of $^{35}SO_2$ Sulfur and $^{14}CO_2$ Carbon by Native Mixed-Grass Prairie and Possible O_2 Effects . . . 441
- Simulation Models: Sulfur Cycle and Ecosystem Level Model 466
- Decomposition-Litter Biomass Dynamics 467

14 The Effects of "Low Level SO_2" Exposure on Sulfur Accumulation and Various Plant Life Responses of Some Major Grassland Species on the ZAPS Sites
P. M. Rice, L. H. Pye, R. Boldi, J. O'Loughlin, P. C. Tourangeau, and C. C. Gordon 494

- Sulfur Accumulation in Plants 508
- Washing Experiment 531
- Fluoride Uptake 532
- Vegetative Phenology 532
- Seed Work 541
- Mycorrhizal Studies 554

15 Response of Selected Small Grains, Range Grasses and Alfalfa to Sulfur Dioxide
R. G. Wilhour, G. E. Neely, D. E. Weber, and L. C. Grothaus 592

Field Exposure Study 597
Range Grass and Alfalfa Study 603

16 Plant Community Changes Due to Low Level SO_2 Exposure
J. E. Taylor and W. C. Leininger 610

Plant Community Analysis 612
Canopy Coverage 612
Diversity 619
Phenology 626
Lichens 626

17 Effects of Low-Level SO_2 on Two Native Lichen Species
S. Eversman 642

18 Responses of Ground-Dwelling Insects to Sulfur Dioxide
J. J. Bromenshenk 673

19 Effects of Controlled Levels of SO_2 on Invertebrate Consumers
J. W. Leetham, T. J. McNary, and J. L. Dodd 723

Biomass and Population Dynamics of Invertebrate Fauna 724
The Effect of Chronic SO_2 Air Pollution on Acrididae (Grasshoppers) Population Dynamics and Behavior 738
Nematode Populations 759
Tardigrade and Rotifer Populations 761

20 Small Mammal Investigations at ZAPS: Demographic Studies and Responses to Gradient Levels of SO_2
J. D. Chilgren 764

Species Composition and Description 768
Sex and Age Composition 768
Population Characteristics 770
Survival 772
Home Range 773
Home Range and Biomass: Relationships to Population Density 775
Experimental Effects 775

AN EXPERIMENTAL EVALUATION OF THE FATE AND IMPACT OF SELECTED TRACE ELEMENT STACK EMISSIONS IN THE SOIL-PLANT ENVIRONMENT

21 The Fate and Bioenvironmental Impact of Mercury in Soils
E. R. Landa 792

The Volatile Loss of Soil-Applied Inorganic Divalent Mercury 802

The Volatile Loss of Mercury from Soils Amended with Methylmercury Chloride 808
The Retention of Metallic Mercury Vapor by Soils 813
The Effect of Mercuric Chloride on Carbon Mineralization in Soils 818
The Uptake from Soils of Inorganic Divalent Mercury by Plants 820

22 Uptake of Metallic Mercury Vapor by Soils and Various Plant Species
C. L. Browne and S. C. Fang 829

Estimation of Whole-Plant Resistance to Gaseous Exchange 838
Factors Influencing the Uptake of Hg Vapor by Wheat 843
Differential Uptake of Hg Vapor by Plants 850
In Vitro Conversion of Elemental Mercury Vapor by Homogenized Leaves of Various Plant Species 852
Sorption of Mercury Vapor by Soils 856

23 Effects of Stack Emissions Upon Primary Aspects of Photosynthesis and Photosynthetically-Linked Nitrogen Fixation
R. M. Tetley and N. I. Bishop 866

The Action of Oxygen and Select Metabolic Inhibitors on Photosynthesis, Respiration, and Nitrogen Fixation 870
Effects of Trace Elements on Photosynthesis, Nitrogen Fixation, Respiration and Growth in *Anabaena* 878
Effects of Select Elements on Growth of *Salvina* and *Azolla* 887
Inhibition by Select Trace Elements 887

PRELIMINARY SYNTHESIS

24 The Use of Remote Sensing in Evaluating SO_2 Damage to Grasslands
J. E. Taylor, W. C. Leininger, and T. R. Osberg 899

. . . . 904
Aerial Photography 904
Analysis of Photography 904
Densitometric Analysis 910
Vegetation Mapping 910
General Discussion

25 Methodology Development for Siting Power Plants
J. L. Dodd, W. K. Lauenroth, and W. J. Parton 926

LIST OF CONTRIBUTORS

N. L. Abshire
U.S. Department of Commerce
NOAA
Environmental Research Laboratories
Boulder, Colorado 80302

N. I. Bishop
Department of Botany
Oregon State University
Corvallis, Oregon 97331

R. Boldi
Environmental Studies Laboratory
University of Montana
Missoula, Montana 59801

D. V. Bradley, Jr.
U.S. EPA/EMSL
P.O. Box 15027
Las Vegas, Nevada 89114

J. J. Bromenshenk
Environmental Studies Laboratory
University of Montana
Missoula, Montana 59801

C. L. Browne
Department of Agricultural Chemistry
Oregon State University
Corvallis, Oregon 97331

J. D. Chilgren
U.S. EPA/CERL
200 SW 35th Street
Corvallis, Oregon 97330

M. B. Coughenour
Natural Resource Ecology Laboratory
Colorado State University
Fort Collins, Colorado 80521

J. L. Dodd
Natural Resource Ecology Laboratory
Colorado State University
Fort Collins, Colorado 80521

S. Eversman
Department of Biology
Montana State University
Bozeman, Montana 59715

S. C. Fang
Department of Agricultural Chemistry
Oregon State University
Corvallis, Oregon 97331

C. C. Gordon
Environmental Studies Laboratory
University of Montana
Missoula, Montana 59801

L. C. Grothaus
U.S. EPA/CERL
200 SW 35th Street
Corvallis, Orgon 97330

T. L. Gullett
U.S. EPA/CERL
200 SW 35th Street
Corvallis, Oregon 97330

M. D. Kern
Department of Biology
The College of Wooster
Wooster, Ohio 44691

E. R. Landa
Department of Soil Science
Oregon State University
Corvallis, Oregon 97331

W. K. Lauenroth
Natural Resource Ecology Laboratory
Colorado State University
Fort Collins, Colorado 80521

J. J. Lee
U.S. EPA/CERL
200 SW 35th Street
Corvallis, Oregon 97330

J. W. Leetham
Natural Resource Ecology Laboratory
Colorado State University
Fort Collins, Colorado 80521

W. C. Leininger
Department of Animal and Range Sciences
Montana State University
Bozeman, Montana 59715

D. E. Weber
U.S. EPA/CERL
200 SW 35th Street
Corvallis, Oregon 97330

D. L. Wellman
U.S. Department of Commerce
NOAA
Environmental Research Laboratories
Boulder, Colorado 80302

J. P. Wiggins
Department of Biology
The College of Wooster
Wooster, Ohio 44691

R. G. Woodmansee
Natural Resource Ecology Laboratory
Colorado State University
Fort Collins, Colorado 80521

R. G. Wilhour
U.S. EPA/CERL
200 SW 35th Street
Corvallis, Oregon 97330

G. M. Lerfald
U.S. Department of Commerce
NOAA
Environmental Research Laboratories
Boulder, Colorado 80302

J. C. McFarlane
U.S. EPA/EMSL
P.O. Box 15027
Las Vegas, Nevada 89114

T. J. McNary
Natural Resource Ecology Laboratory
Colorado State University
Fort Collins, Colorado 80521

G. T. McNice
U.S. Department of Commerce
NOAA
Environmental Research Laboratories
Boulder, Colorado 80302

G. E. Neely
U.S. EPA/CERL
200 SW 35th Street
Corvallis, Oregon 97330

J. O'Loughlin
Environmental Studies Laboratory
University of Montana
Missoula, Montana 59801

T. R. Osberg
U.S. EPA/EPIC
Vint Hills Farm Station
Box 1587
Warrenton, Virginia 22186

W. J. Parton
Natural Resource Ecology Laboratory
Colorado State University
Fort Collins, Colorado 80521

E. M. Preston
U.S. EPA/CERL
200 SW 35th Street
Corvallis, Oregon 97330

R. F. Pueschel
U.S. Department of Commerce
NOAA
Environmental Research Laboratories
Boulder, Colorado 80302

L. H. Pye
Environmental Studies Laboratory
University of Montana
Missoula, Montana 59801

P. M. Rice
Environmental Studies Laboratory
University of Montana
Missoula, Montana 59801

R. D. Rogers
U.S. EPA/EMSL
P.O. Box 15027
Las Vegas, Nevada 89114

J. E. Taylor
Department of Animal and Range Sciences
Montana State University
Bozeman, Montana 59715

R. M. Tetley
Department of Botany
Oregon State University
Corvallis, Oregon 97331

S. K. Thompson
U.S. EPA/CERL
200 SW 35th Street
Corvallis, Oregon 97331

G. L. Thor
Natural Resource Ecology Laboratory
Colorado State University
Fort Collins, Colorado 80521

P. C. Tourangeau
Environmental Studies Laboratory
University of Montana
Missoula, Montana 59801

C. C. Van Valin
U.S. Department of Commerce
NOAA
Environmental Research Laboratories
Boulder, Colorado 80302

D. B. Weber
U.S. EPA/CERL
200 SW 35th Street
Corvallis, Oregon 97330

ACKNOWLEDGEMENTS

Many individuals have contributed to the preparation of this document. The editorial assistance of Susan With, Karen Randolph and others is much appreciated.

Our work could not proceed without the help and support of the people of southeastern Montana, especially the ranchers on whose land we are working and the personnel and persons residing at and near Fort Howes Ranger Station, Custer National Forest. The Kluver's and the McRae's have been particularly supportive.

ECOLOGICAL EFFECTS MONITORING IN THE COLSTRIP VICINITY

SECTION 1

AEROSOL CHARACTERIZATION IN THE VICINITY OF COLSTRIP, MONTANA

C. C. Van Valin, R. F. Pueschel, D. L. Wellman,
N. L. Abshire, G. M. Lerfald, and G. T. McNice

ABSTRACT

Both natural aerosols and those generated by the coal-fired power plant at Colstrip, Montana, were studied over a three-year period from 1975, before the power plant was operational through 1977 when it was in full operation. A multi-sensor approach was employed which included complete particle and gas analysis from ground-based and airborne laboratories supported by a variety of remote sensing devices (laser radar, acoustic sounder, and solar photometry). The background aerosols were found to consist of irregular-shaped particles, primarily alumino-silicates with relatively high concentrations of the metallic elements for a rural area. Aerosols generated by the power plant were relatively large (approximately 1 μm), glassy, alumino-silicate spheres with a high incidence of calcium and sulfur when collected in the plume near the power plant, becoming smaller (<.4 μm) sulfur-containing spheres at large distances downwind. The elemental shift to sulfur is attributed to a gas-to-particle conversion process. These artificial aerosols increased the available ice-nuclei concentration by an order of magnitude, which could be significant to the formation and distribution of precipitation. As the plume traveled downwind, even under a stable temperature inversion layer, it was found to break up into inhomogeneous parcels.

INTRODUCTION

This program is designed to characterize the aerosol content of the Colstrip power plant plume and to aid the U.S. Environmental Protection Agency at Corvallis, Oregon, in the assessment of its impact on the ecosystem. Particles emitted by coal-fired plants (*e.g.*, ash), or formed from gases emitted (*e.g.*, sulfates or chlorides) produce an array of effects on the ecosystem. They may produce direct biological effects through contact with leaves and soil; they may affect solar radiation reaching the biosphere by absorption and blanketing mechanisms, thus altering surface temperatures. Aerosol properties such as solubility, crystalline formation, size, shape, surface structure, and chemical composition will bear on the formation and distribution of rain, snow, and hail, either near the source or far downwind, depending on the temperature structure (inversions) and on mixing and diffusion characteristics of the atmosphere downwind from the source.

Small particles are an especially significant part of the pollution emitted by fossil fuel power plants, even when the stack emission has been carefully filtered (we exclude, for this report, water droplet clouds and fogs from the term aerosols). Aerosols may range from a cluster of a few molecules to particles several tens of micrometers across. The larger aerosol particles fall out rapidly and are not frequently observed in the atmosphere except in the immediate vicinity of their source. However, particles up to several micrometers in diameter may remain suspended in the atmosphere for long periods, up to weeks or months. Thus, if stack filters are not effective in removing small particles, the plume may produce significant pollution which may accumulate in the vicinity, or may be swept by winds over long distances.

MATERIALS AND METHODS

In order to determine the amount of such material, its source, its distribution in the atmosphere, its composition, its size and shape distributions, the effects of weather conditions on its formation and its effect on the high plains heat budget, it was necessary to use a combination of remote, *in-situ* and airborne sensors, before and after contamination by the power plant, and over sufficient time to obtain representative averages. These sensors were contained in a mobile atmosphere characterization laboratory, an airborne atmospheric characterization laboratory, and a mobile remote sensing laboratory, all of which were described in detail in the Third Interim Report (Abshire *et al.*, 1978).

The observation schedule was established to bracket the high plains growing season each year with two intensive measurement periods, roughly centered about June 1 and September 1. The specific intervals are:

Year	Spring	Fall
1975	18 May - 15 June	17 Aug - 15 Sept
1976	21 May - 5 June	20 Aug - 11 Sept
1977	21 May - 4 June	20 Aug - 4 Sept

During 1975, the power plant was not in operation so that this data represents clear air baseline, as previously described (Abshire *et al.*, 1978). In 1976, the plant was in intermittent operation, and in 1977, it was in full operation.

The ground-based equipment was located at Hay Coulee, 12 kilometers southeast of Colstrip, during most of the measurement periods, the only exception occurring during the second week of the fall 1977 experiment, when the remote sensing laboratory was moved to the top of a butte, four kilometers southeast of the plant (the Battelle No. 1 site).

The airborne laboratory recorded data over most of the area within a 50-kilometer radius of the power plant, either operating in the plume or in response to the requirements of the two ground-based laboratories.

RESULTS AND DISCUSSION

Atmospheric Characterization Laboratories

The aerosol content of the plume and its vicinity was classified according to number, size, shape and elemental composition. This classification permits distinction between combustion-produced aerosols, which for energetic reasons must be small and spherical, and naturally-formed particles that need not be spherical, and that have a preferred size range of 0.6 to 1.0 μm diameter. Furthermore, the elemental composition of the particles hints toward the types of compounds that are formed and their water solubility. These properties determine the effects of the aerosol on visibility, on the colloidal stability of clouds, and eventually on amounts and frequency of precipitation.

Parameters Measured

Measurements of meteorological and aerosol parameters begun during the two 1975 observation periods at Hay Coulee were continued. Wind direction and velocity, temperature, relative humidity, light scattering coefficient (b_{scat}), total aerosol population (Aitken nuclei (AN)) and solar energy received, were measured continuously. Nuclepore membrane filters were collected at specified times; ice nuclei (IN) collected on the filter were measured in a thermal diffusion chamber and selected filters were examined in the scanning electron microscope with an energy dispersive x-ray accessory (SEM-EDX) to determine the elemental composition of individual particles. The acoustic ice nucleus counter was also utilized to measure IN concentrations. Cloud condensation nuclei (CCN) were measured at specified times with the thermal diffusion chamber-photographic method.

Instruments carried by the aircraft, a Cessna 206 six-place single engine, high-wing airplane, were utilized to measure temperature, relative humidity, AN, b_{scat}, ozone (O_3), nitric oxide (NO), nitrogen dioxide (NO_2), and sulfur dioxide (SO_2) continuously during the flight. Filter samples were collected for subsequent analysis for IN and for particulate properties by SEM-EDX. CCN were measured by the thermal diffusion-photographic method.

Observations

The Colstrip power plant was in limited operation for only two or three days during the period 21 May - 5 June 1976. In consequence, the measurements mainly reiterated the findings of the 1975 field periods (Schnell *et* *al.*, 1976; Parungo *et al.*, 1978; Pueschel *et al.*, 1974; Van Valin *et al.*, 1976), *i.e.*, the natural aerosol is relatively abundant in the metallic elements and is therefore slightly more effective in the ice nucleation than is the natural aerosol in most other locations that we have investigated; the CCN concentration is generally within the range of 500-1000 cm^{-3}, and b_{scat} is typical of clean environments.

The period 28 August - 11 September 1976 was dry, with warm days and cool nights. During the night the winds were light and variable; daytime experienced wind velocities of 2 to 6 m sec^{-1}, predominantly westerly. Relative humidity increased to maxima just before sunrise, typically reaching 80 percent. Coincident with the maxima in relative humidity were maxima in b_{scat}. During eleven successive 24-hour periods, the average maximum was $1.27 \times 10^{-4}m^{-1}$ and the average minimum was $0.36 \times 10^{-4}m^{-1}$; b_{scat} was, on the average, 3.5 times greater during the time of highest relative humidity than during the period of lowest relative humidity (Figure 1.1a). It is not reasonable to believe that the aerosol character changed from night to day, and this is therefore interpreted as evidence of a rather hygroscopic aerosol population, with optically effective particle size increasing, during periods of higher humidity, from absorption of water. This is supported by the finding of high concentrations of Cl- and S-containing particles (Figure 1.2). Both elements form anions that can lead to water soluble substances. The day-to-night variation in b_{scat} was much less extreme during the two study periods of 1977, being about a factor of two, on the average, with approximately equal minimum values. Figures 1.1a, b, and c are the record for the continuously measured parameters during 20 August - 10 September 1976, 20 May - 4 June 1977, and 20 August - 3 September 1977, respectively.

Figures 1.2 and 1.3 are derived from SEM-EDX examination of filter samples collected in the mobile laboratory at Hay Coulee and with the airborne laboratory in the vicinity of Colstrip and Hay Coulee on May 27 and 29, 1977. On May 27, Hay Coulee experienced a fairly intense plume fumigation, as shown by the increase in AN to $6.0 \times 10^{4}cm^{-3}$, as compared to a background concentration of $3.2 \times 10^{3}cm^{-3}$ (Figure 1.1b). Hay Coulee had not, to our knowledge, experienced any plume exposure on May 29, 1977. Therefore, samples were collected from these two days that were to provide pre-exposure and during-exposure samples on the day of fumigation, plus samples collected at similar times-of-day on a non-exposure day, as well as some aircraft samples over Hay Coulee and in the plume close to Colstrip. In all cases except one, 75 particles were examined on each filter; the May 29 Air #5B sample was so lightly loaded that 75 particles could not be found within the prescribed 1 mm^2 part of the filter. The particles were classified according to diameter in ranges 0.1 - 0.4 μm, 0.6 - 1.0 μm, and 1.5 - 10.0 μm, and according to shape, whether spherical or irregular. As it turned out, this sample selection yielded considerably more information and understanding of plume behavior and composition than we had expected, as well as posing some questions regarding particle origin that we have not yet answered. The May 27 Ground #1 and Air #1 analyses show, because of their similarity, that these samples were collected in the same air mass. An unanswered question in connection with these two samples is: why do these samples contain so many spherical particles, 31 and 45 percent, respectively, when the available

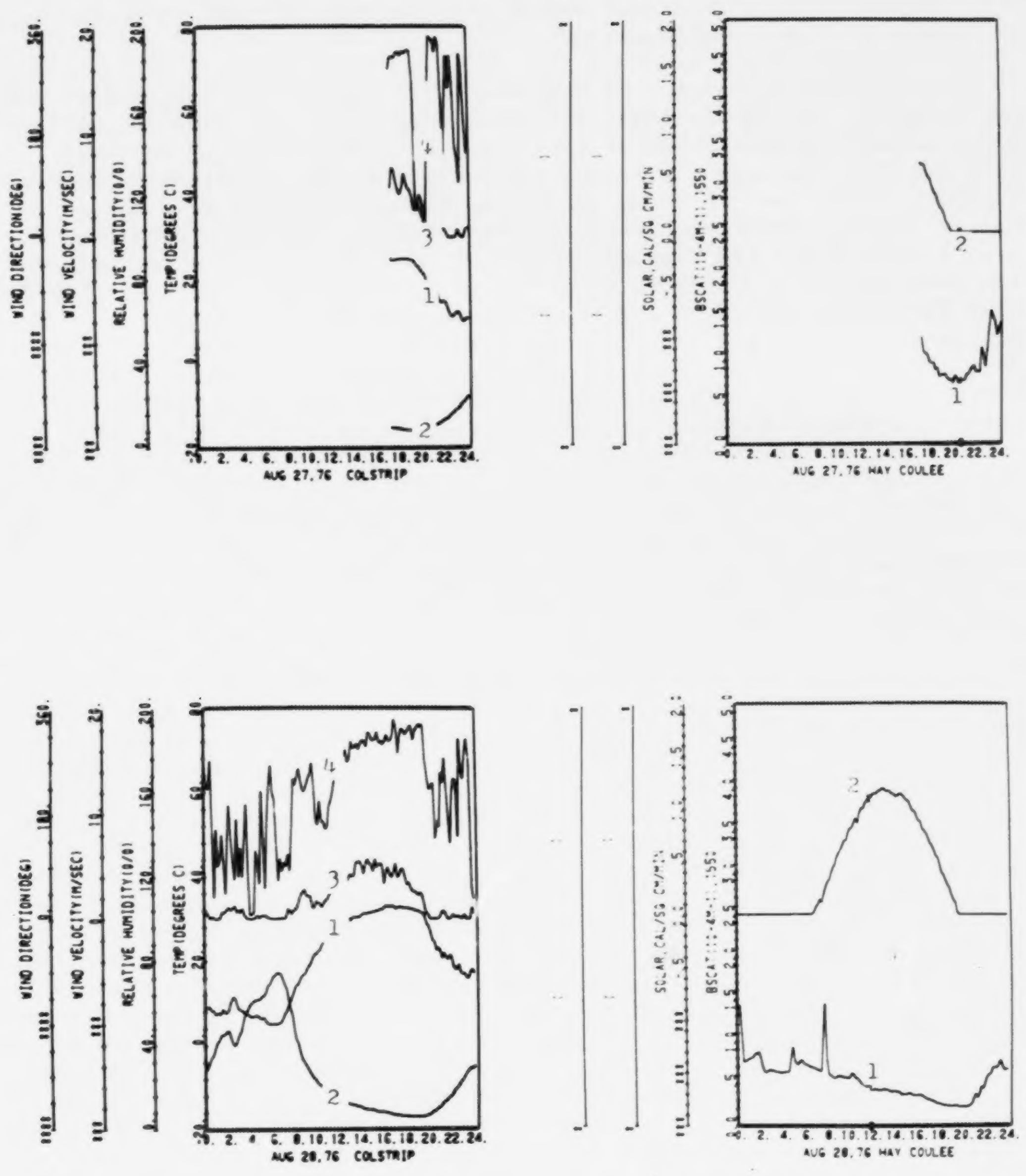


Figure 1.1a. Continuously measured parameters at Hay Coulee, August 27 - September 7, 1976. Reading from right to left, vertical scales on the left of each graph refer to plots numbered 1, 2, 3, and 4 respectively. The horizontal scale refers to Mountain Daylight Time.

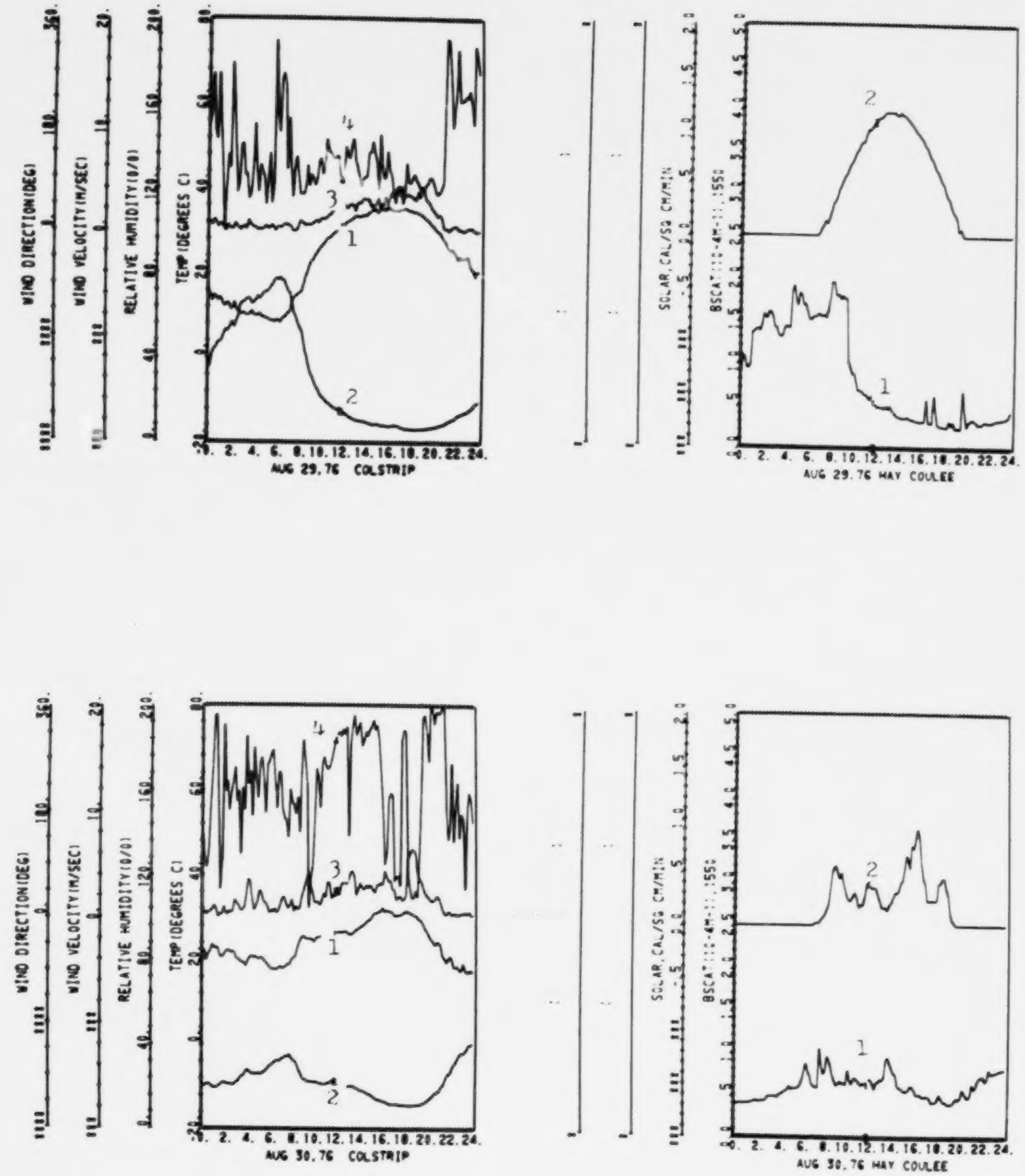


Figure 1.1a. Continued.

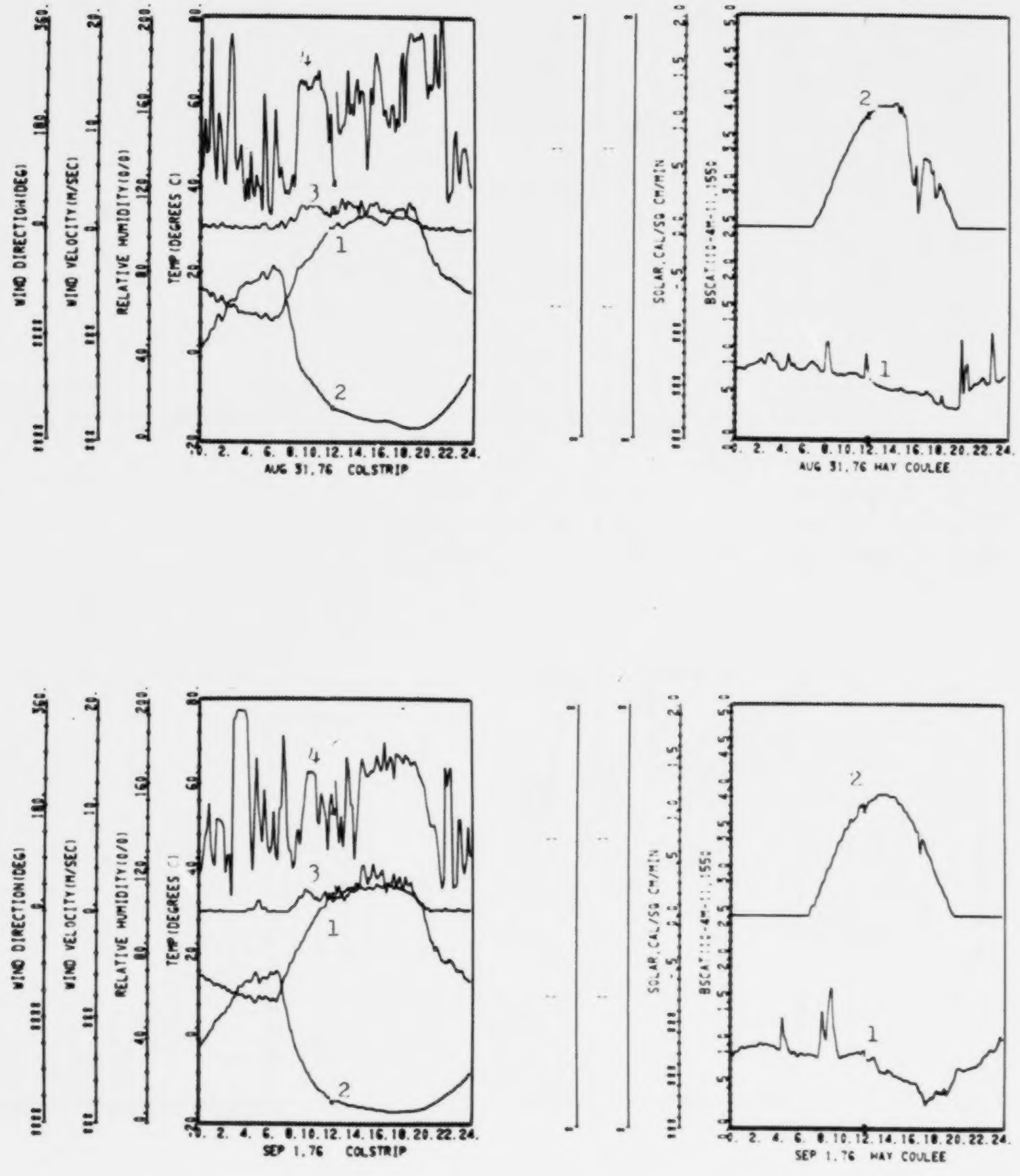


Figure 1.1a. Continued.

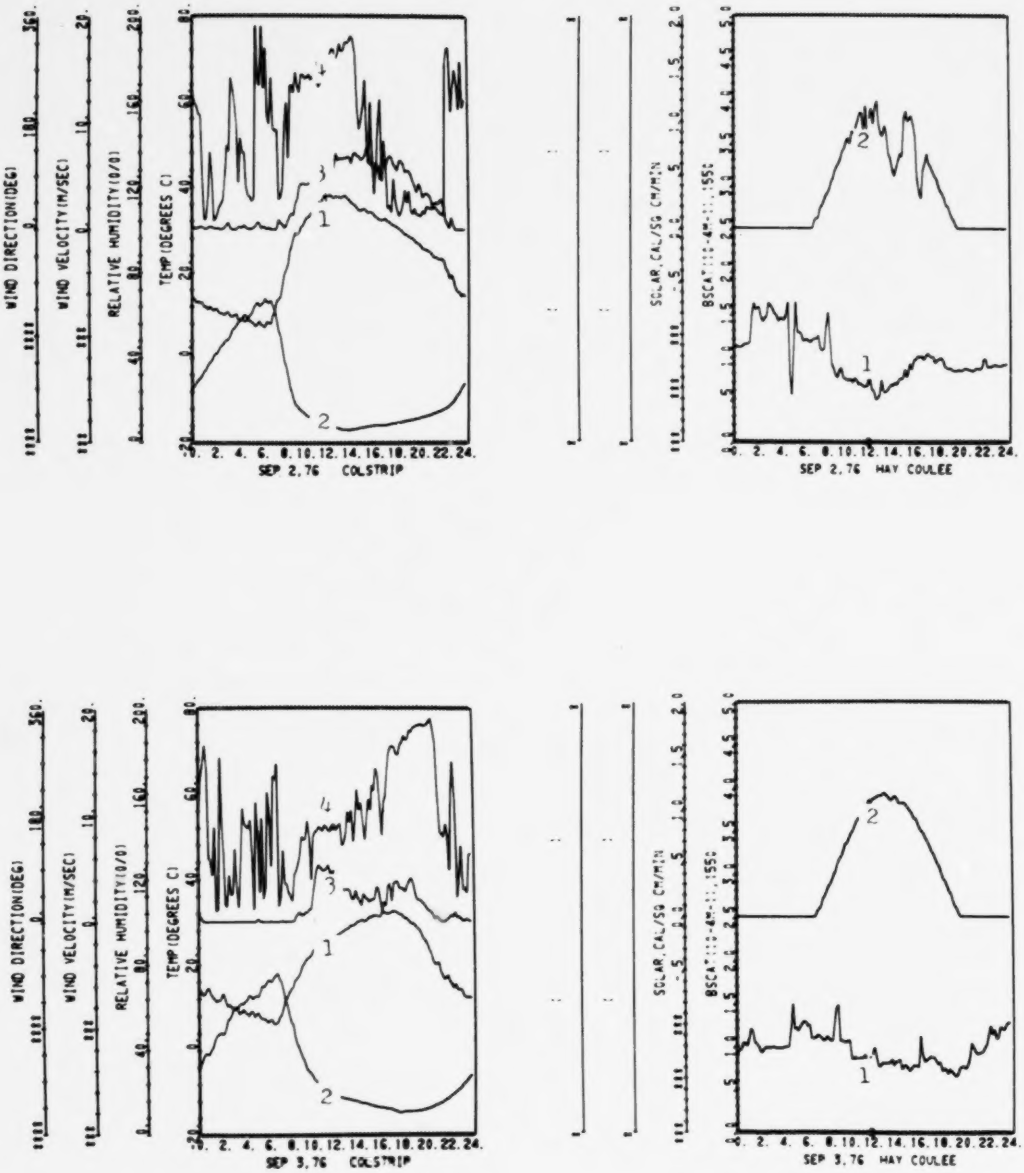
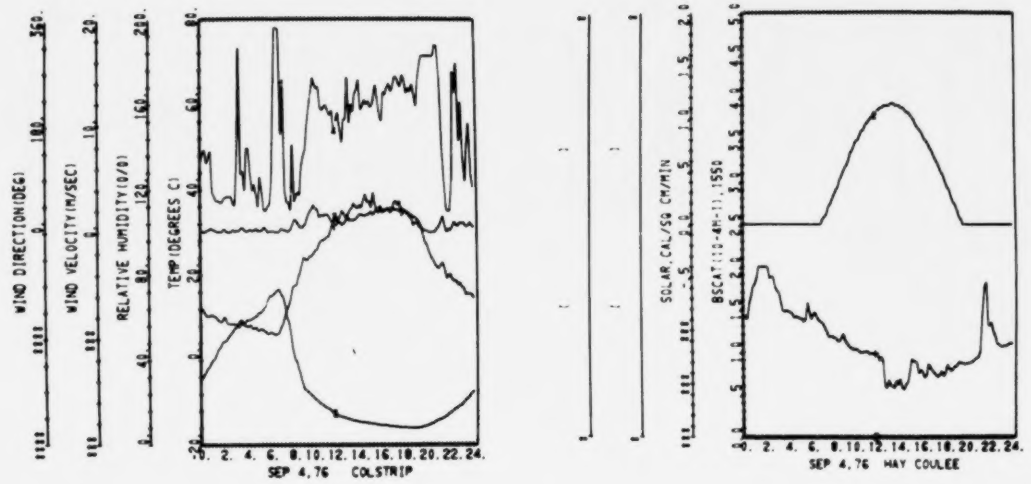


Figure 1.1a. Continued.



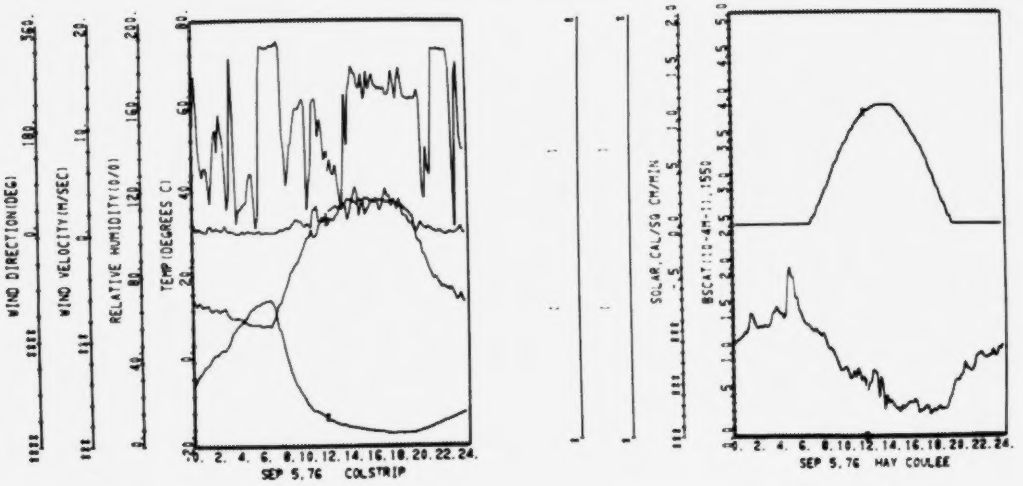


Figure 1.1a. Continued.

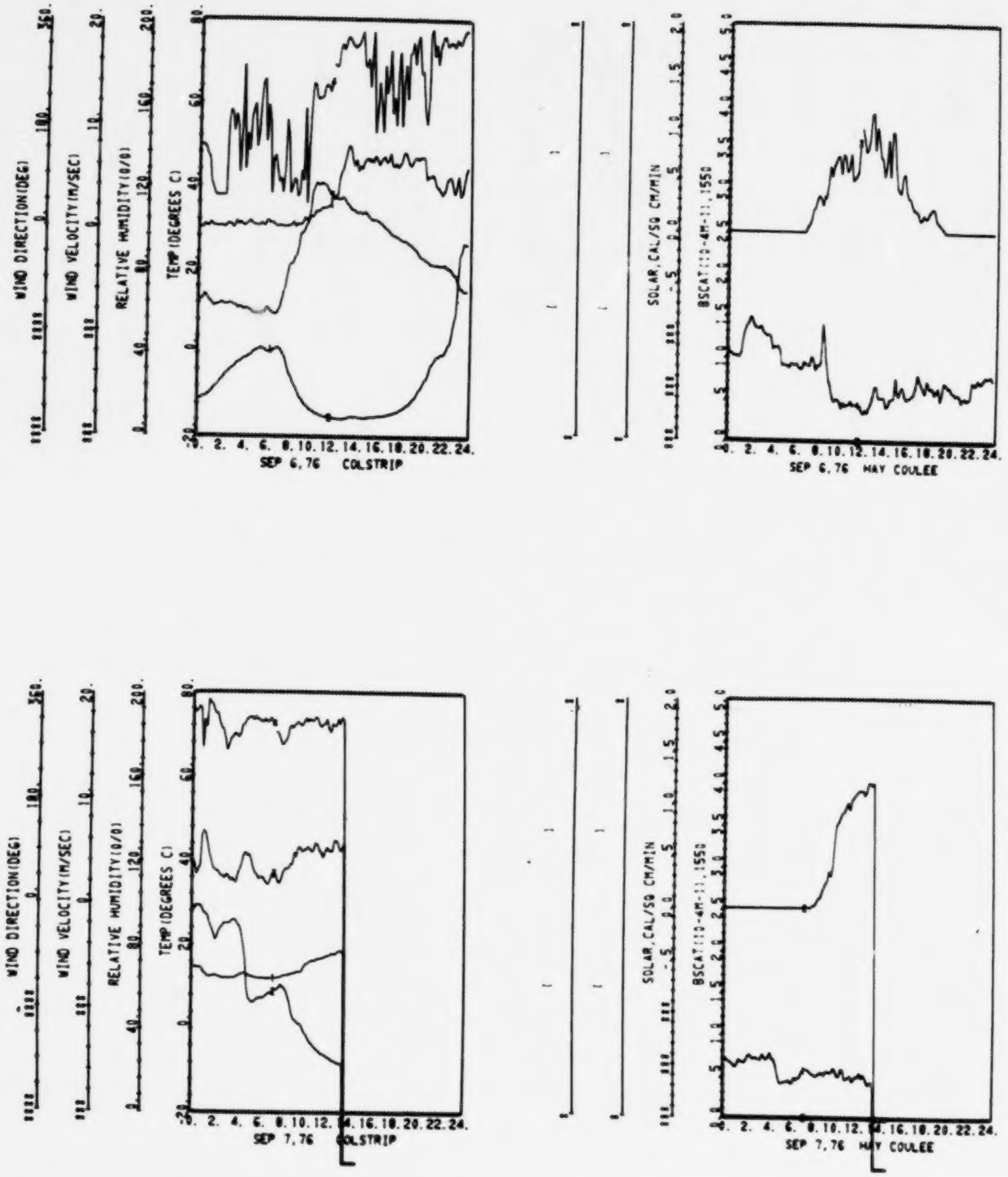


Figure 1.1a. Continued.

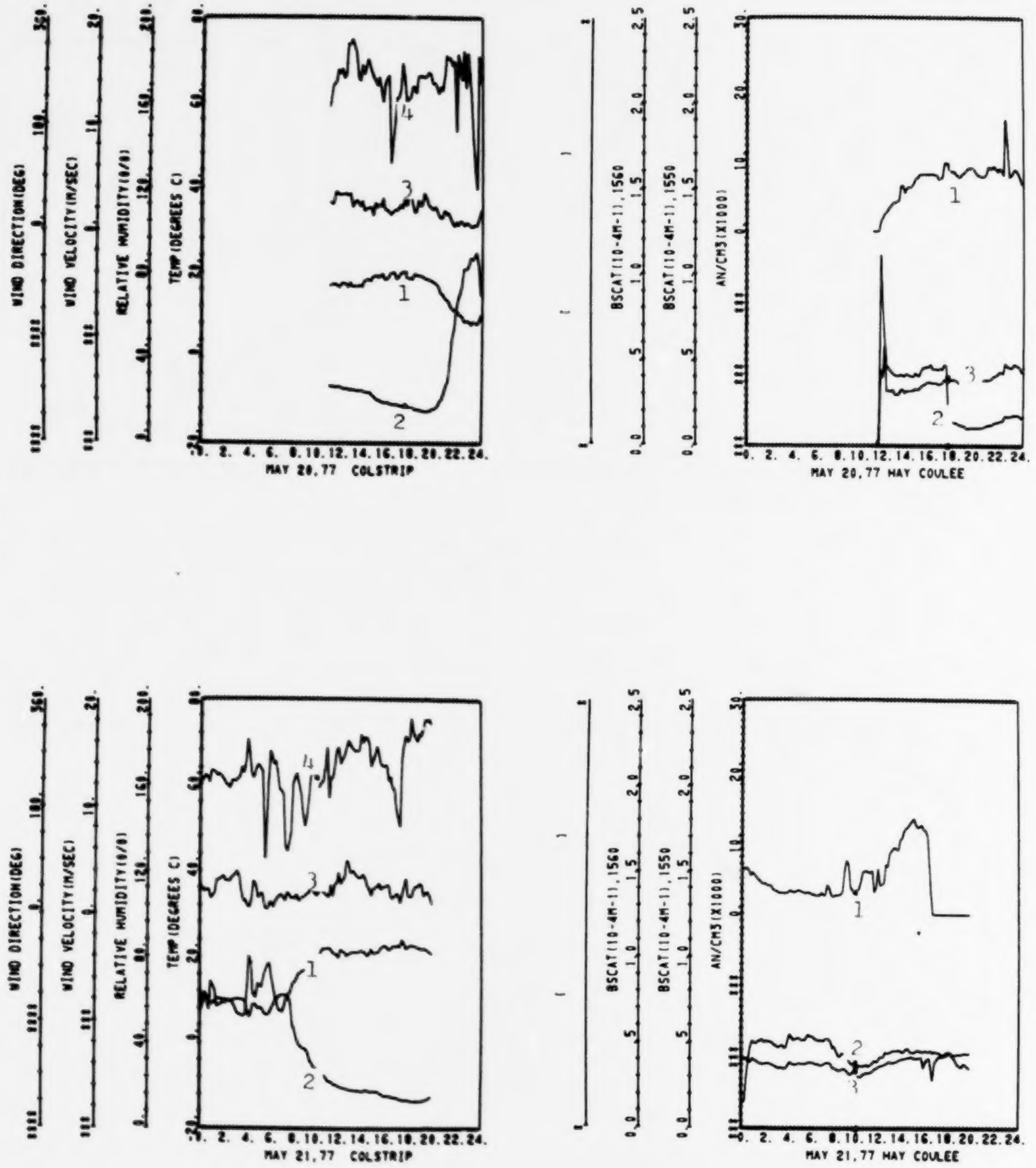


Figure 1.1b. Continuously measured parameters at Hay Coulee, May 20 - June 4, 1977. Reading from right to left, the vertical scales on the left of each graph refer to plots numbered 1, 2, 3, and 4 respectively. The horizontal scale refers to Mountain Daylight Time.

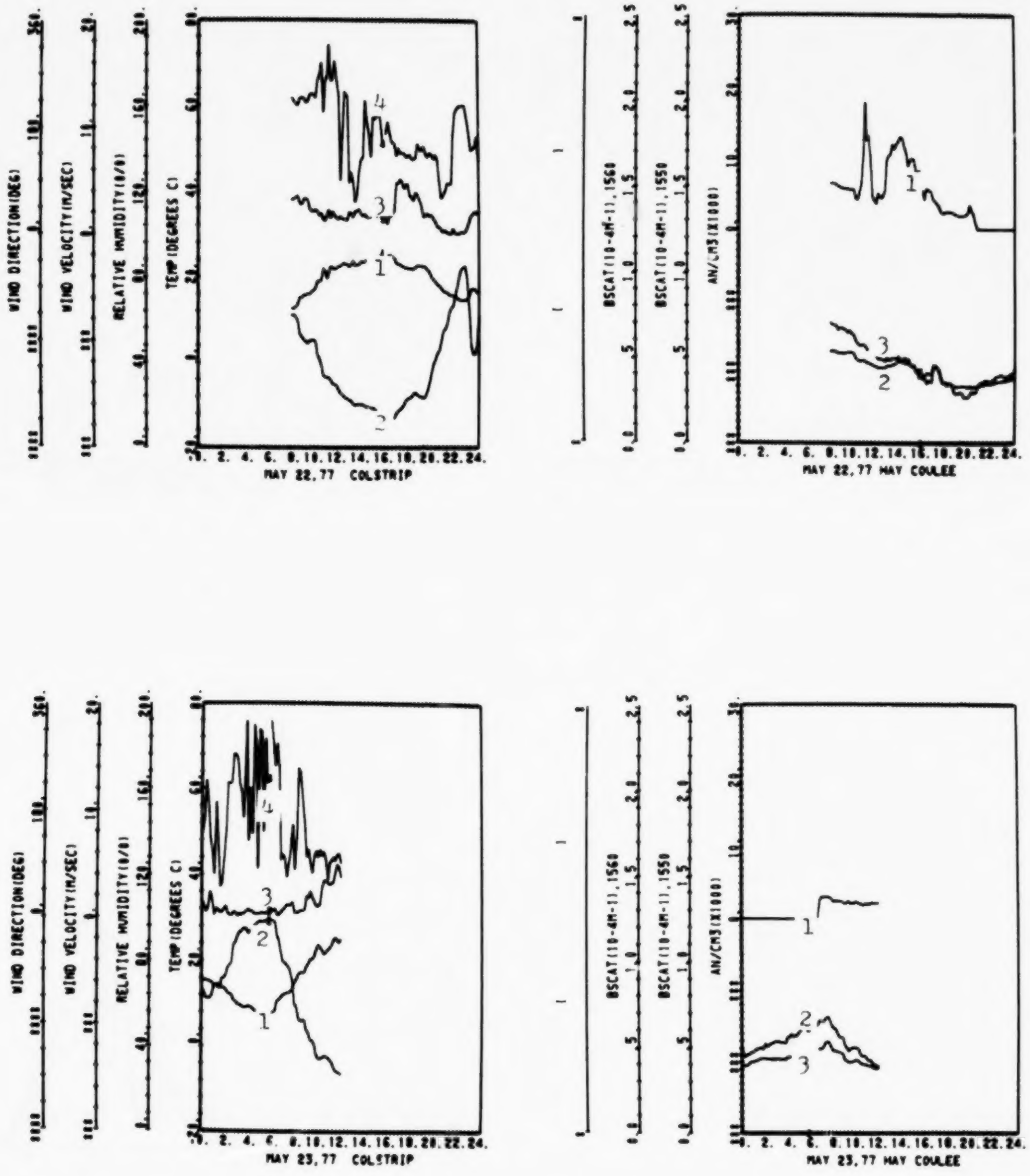


Figure 1.1b. Continued

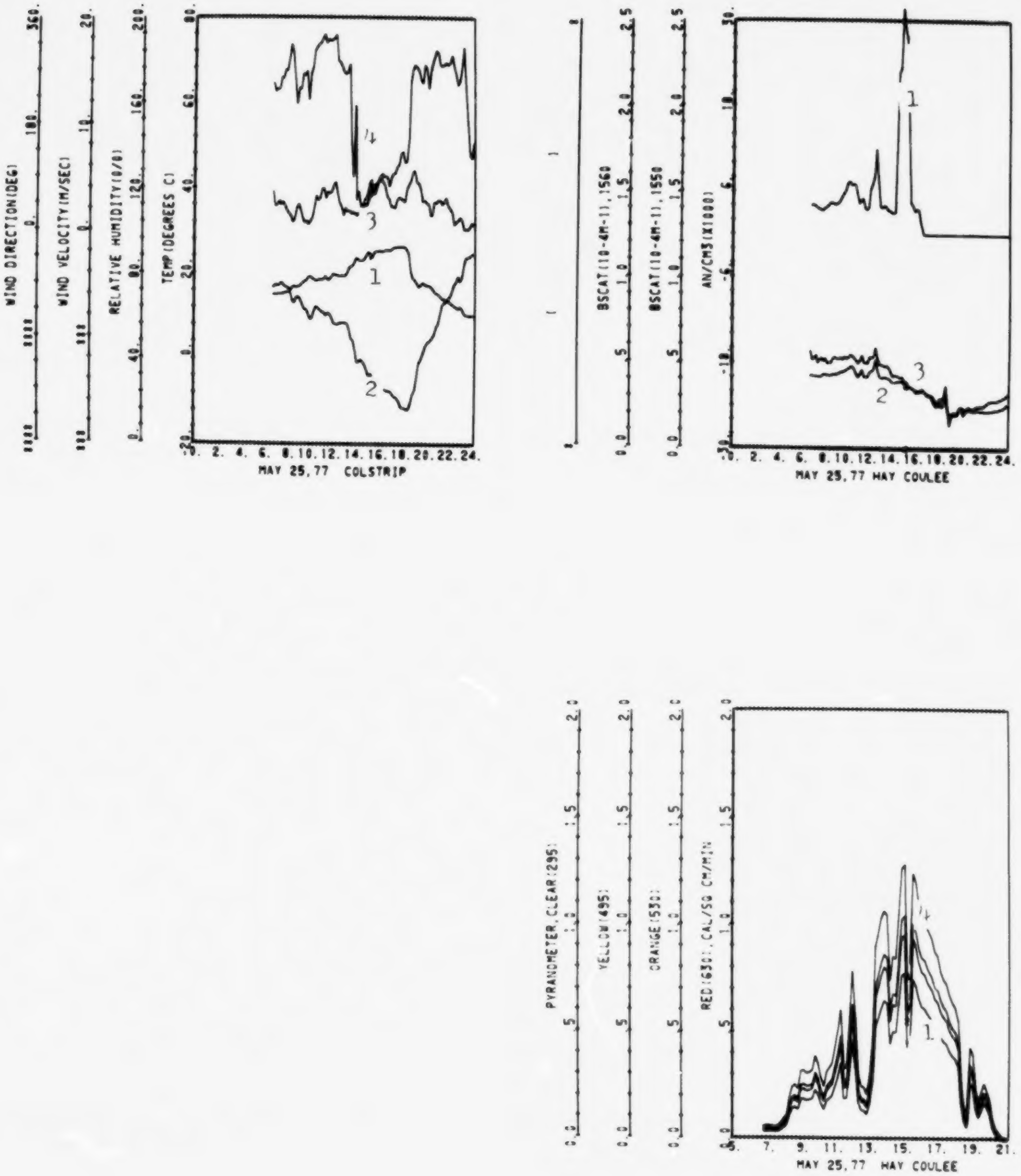


Figure 1.1b. Continued.

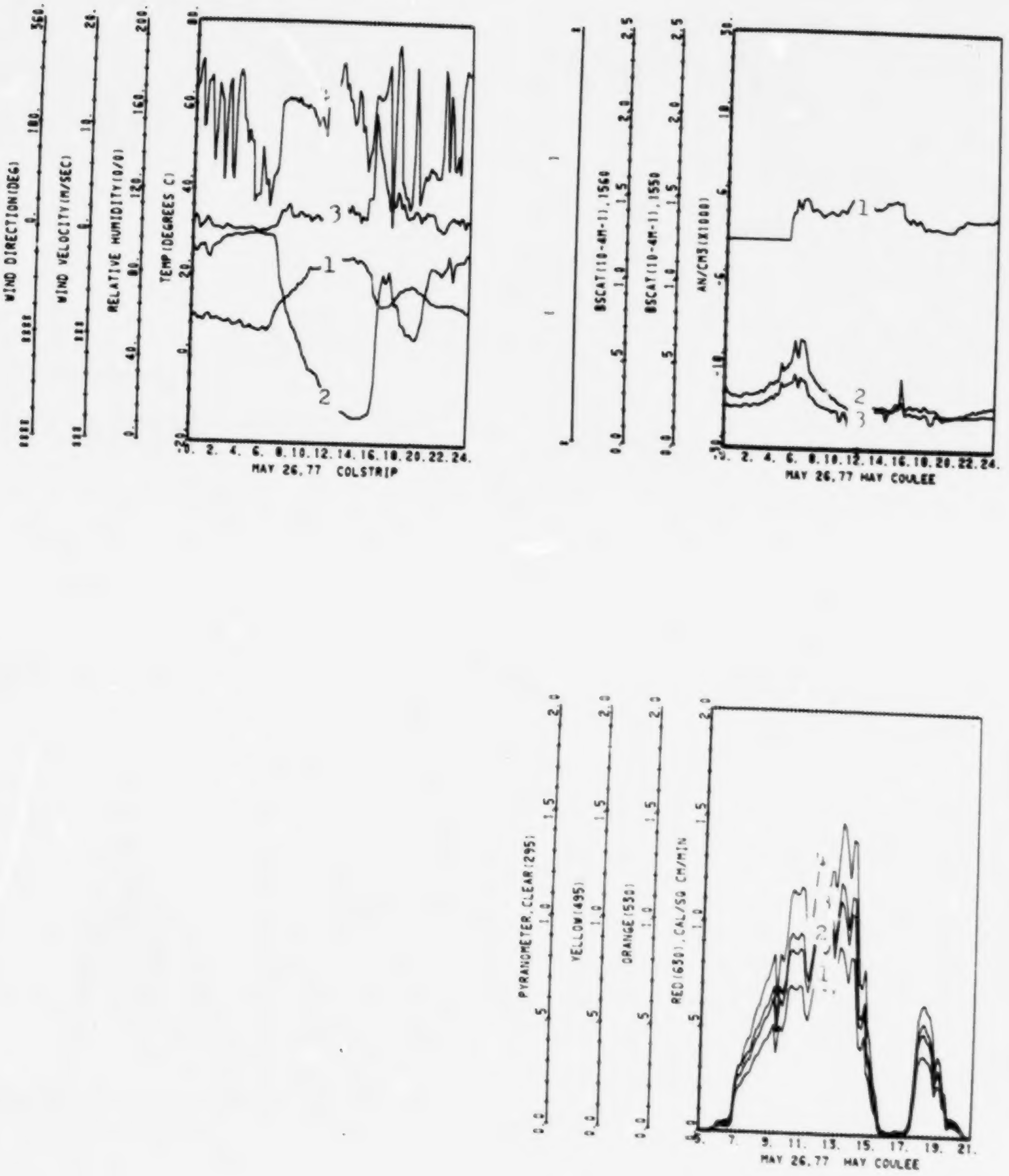


Figure 1.1b. Continued.

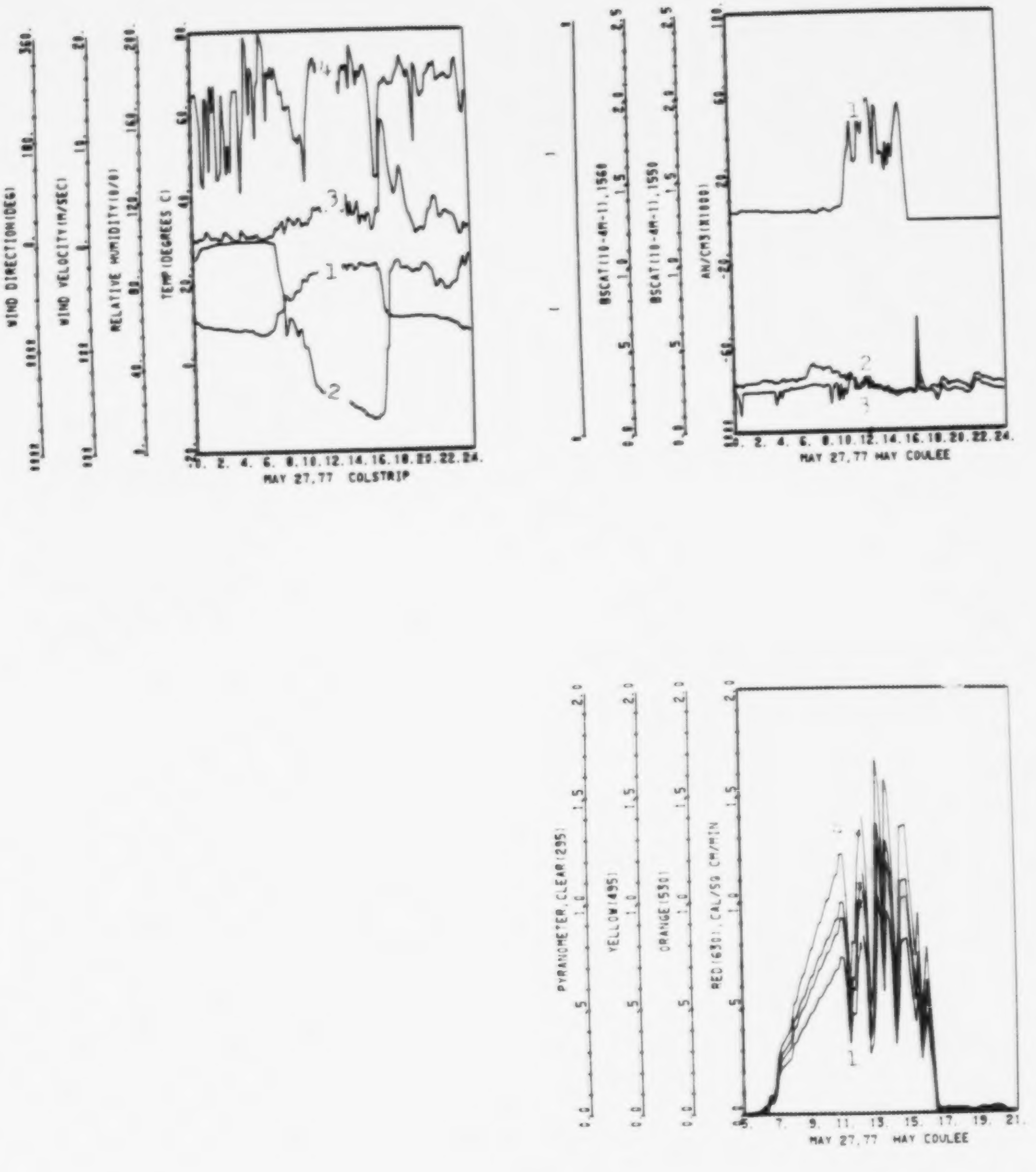
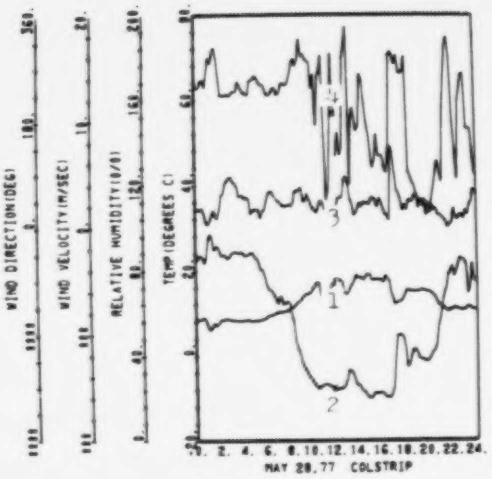
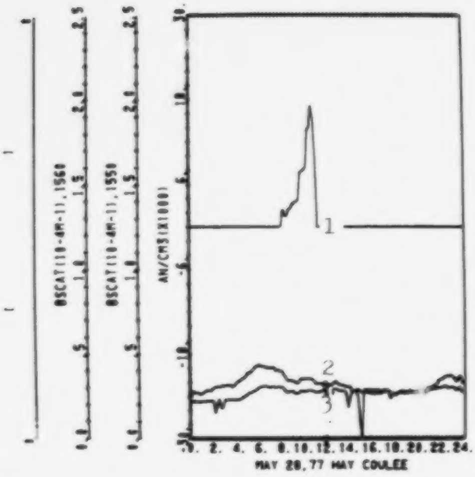


Figure I.1b. Continued





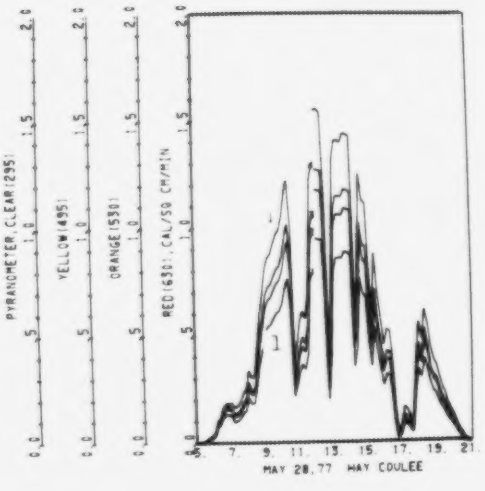


Figure 1.1b. Continued.

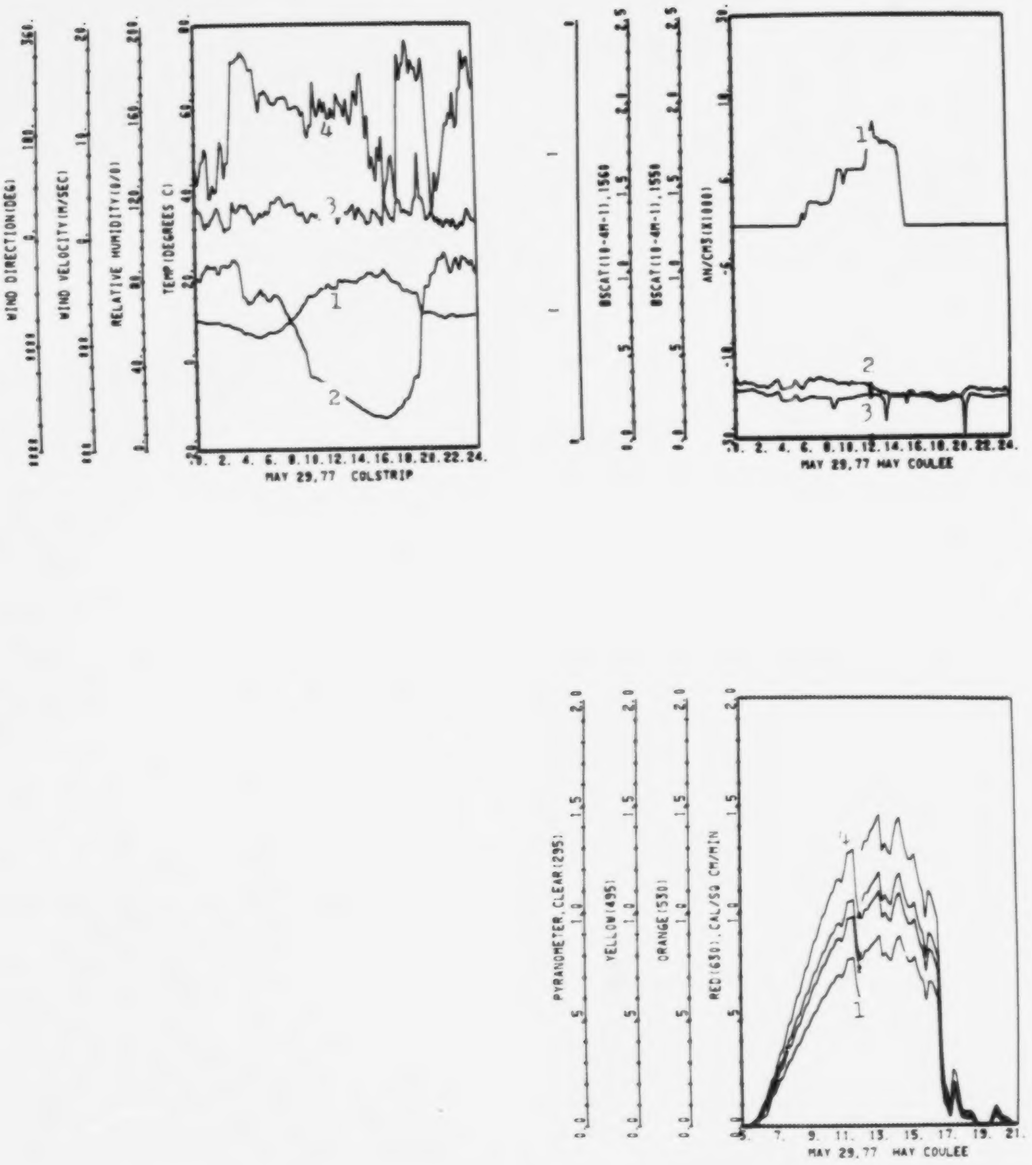


Figure 1.1b. Continued.

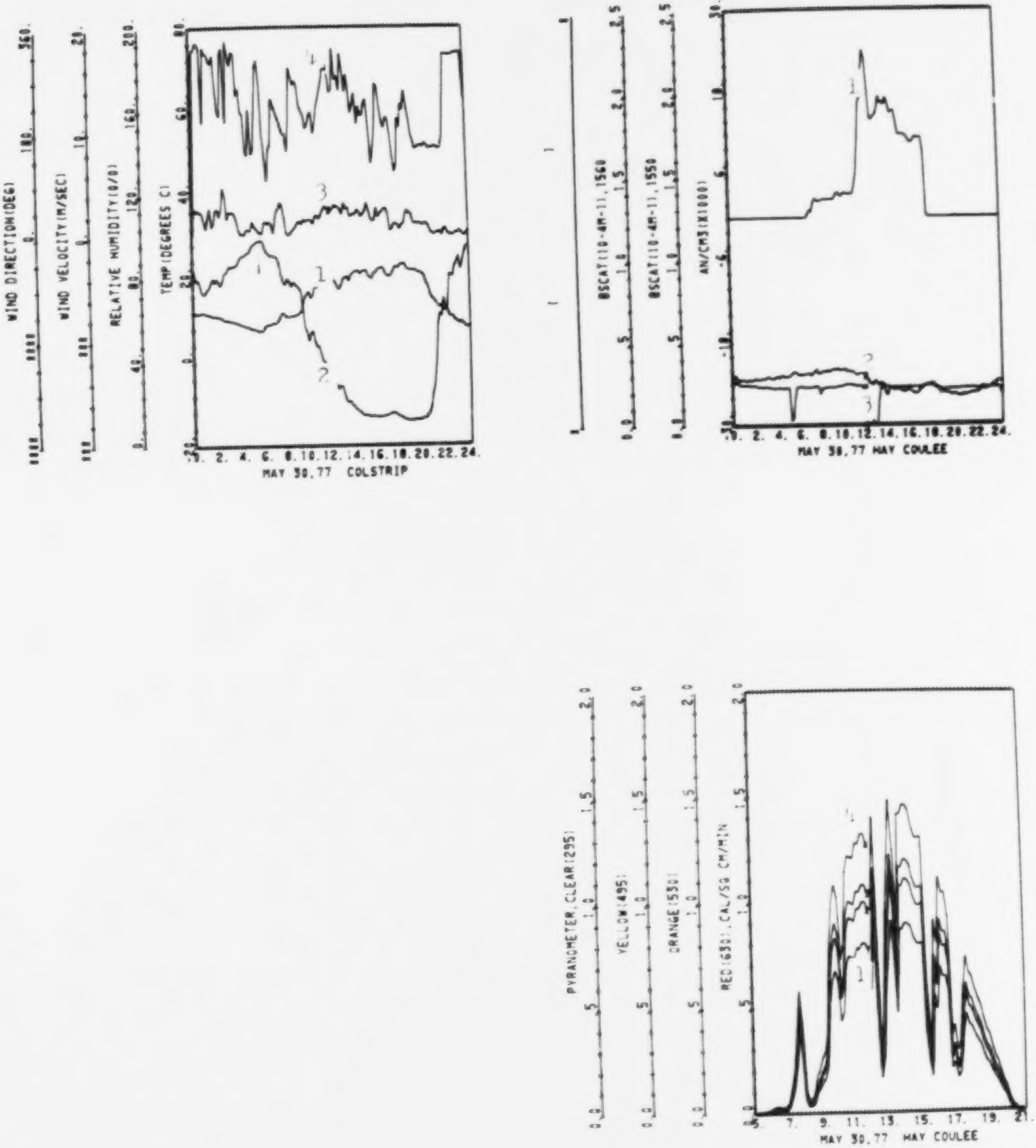


Figure 1.1b. Continued.

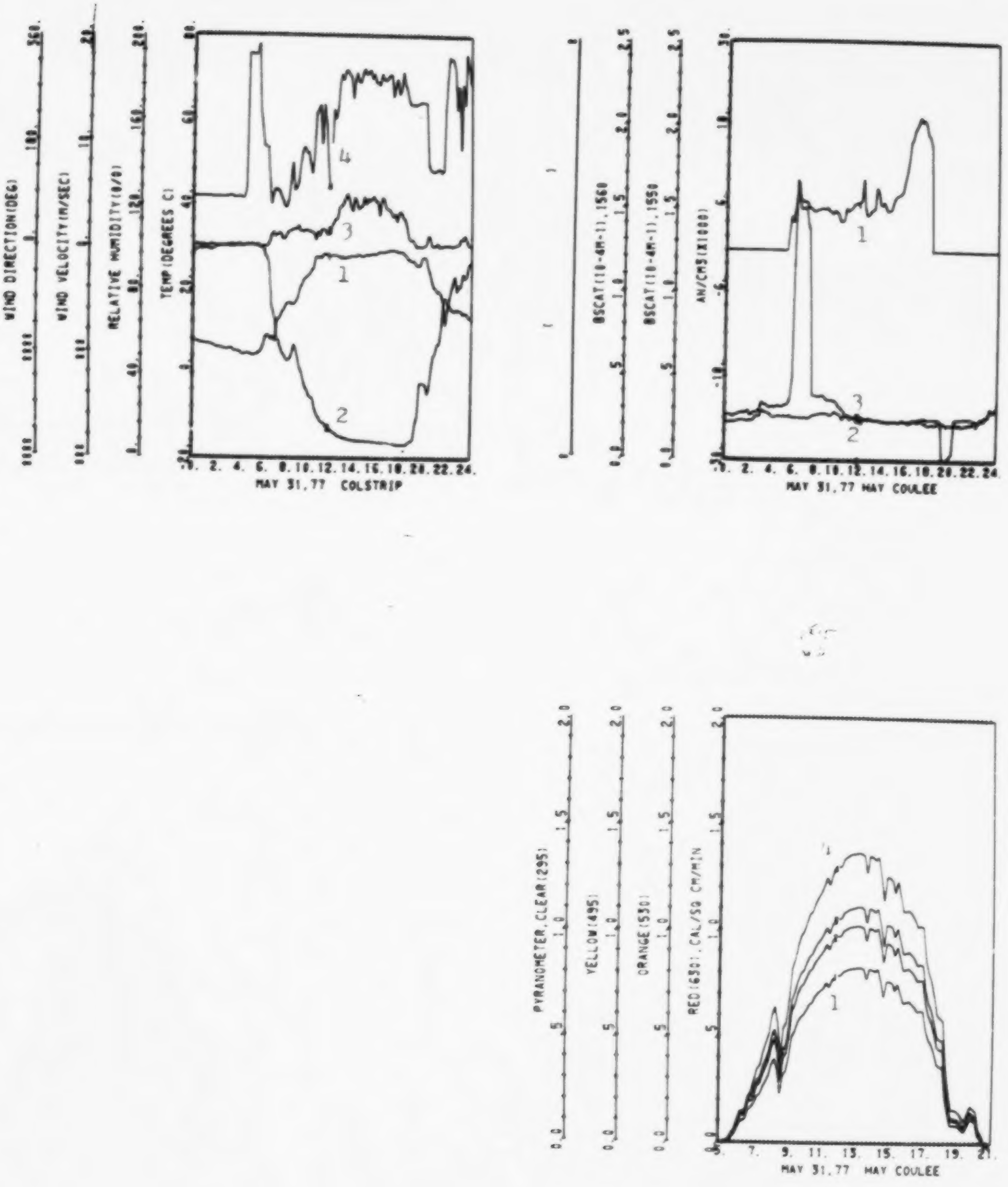


Figure 1.1b. Continued.

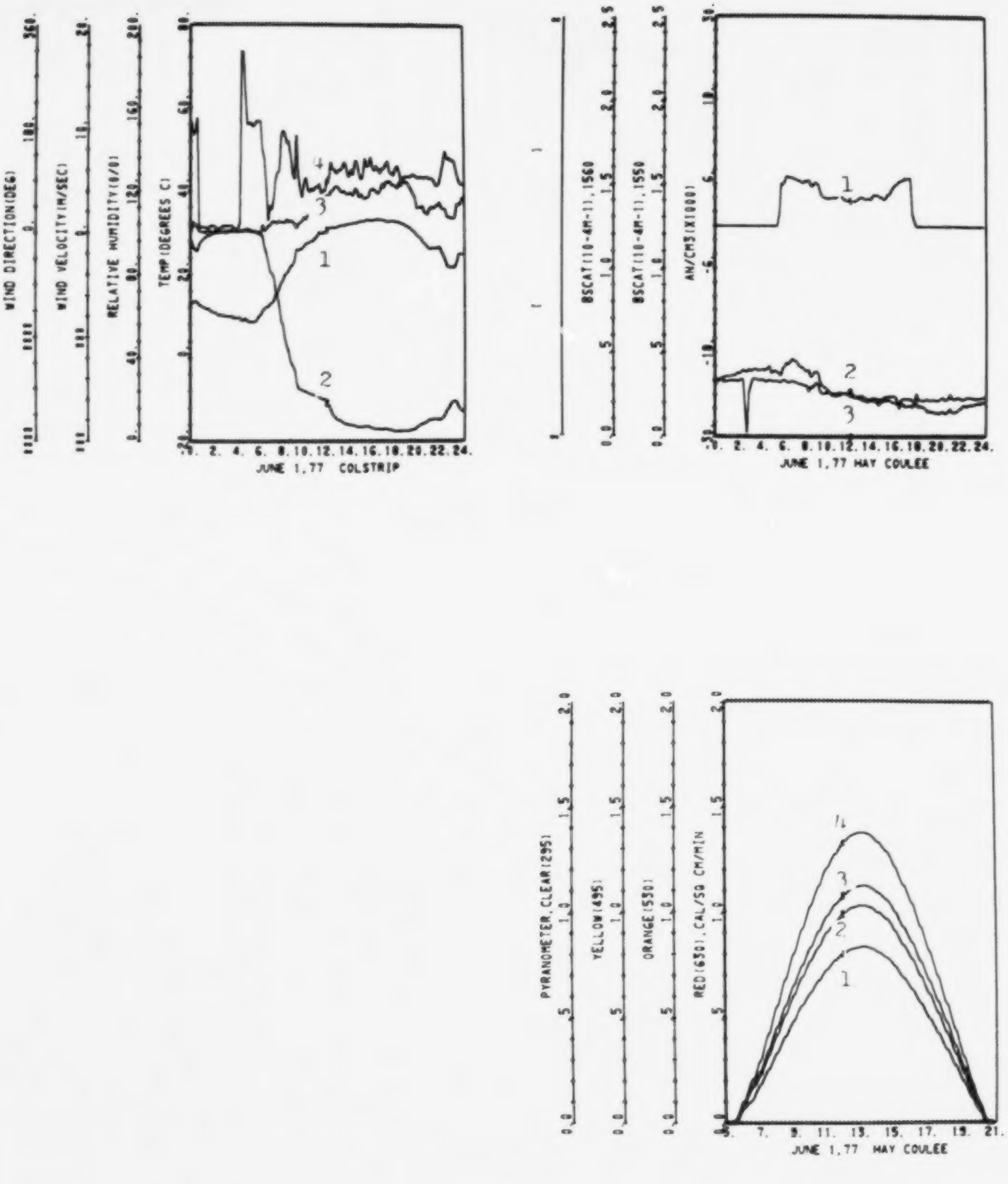


Figure 1.1b. Continued.

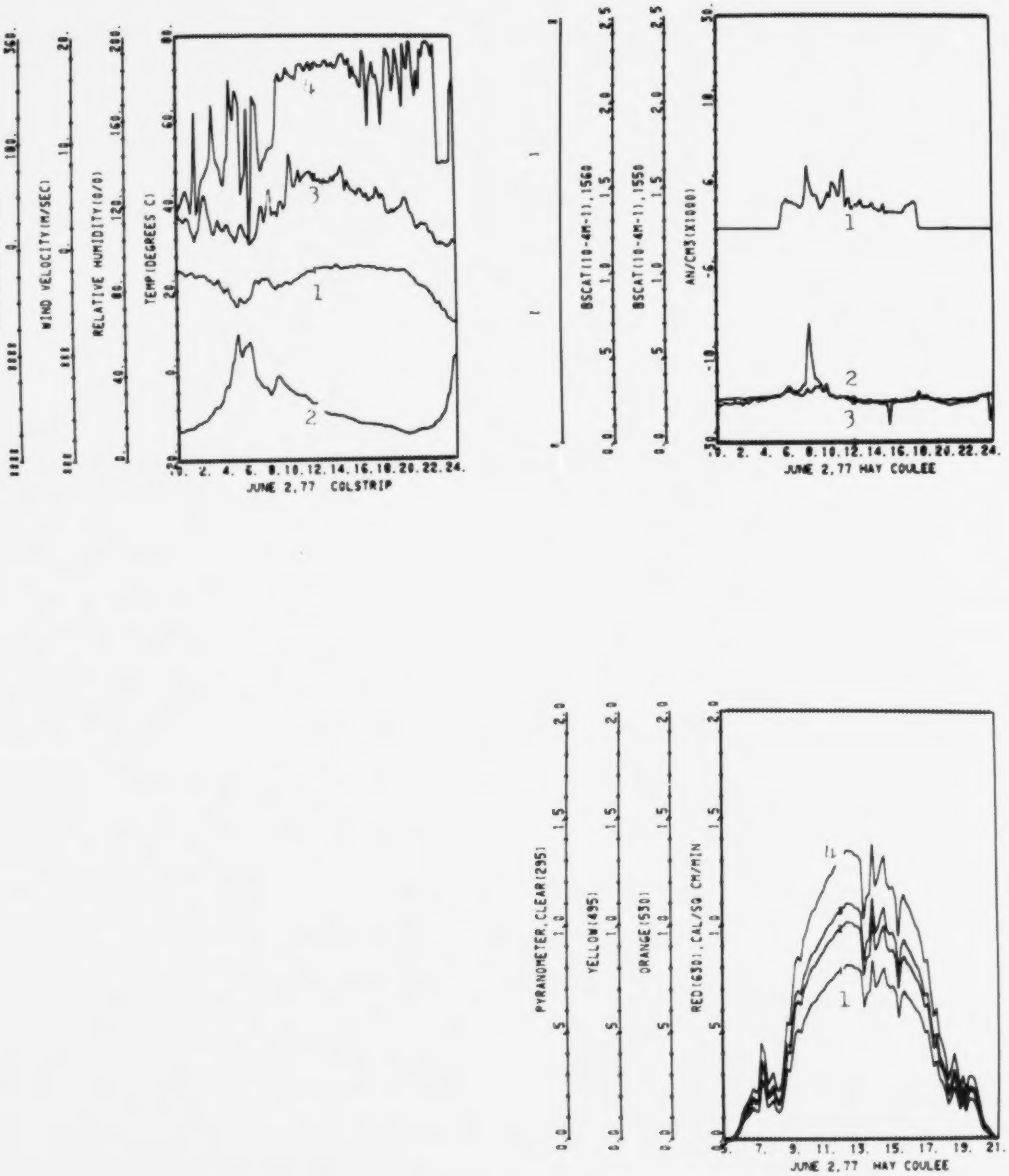


Figure 1.1b. Continued.

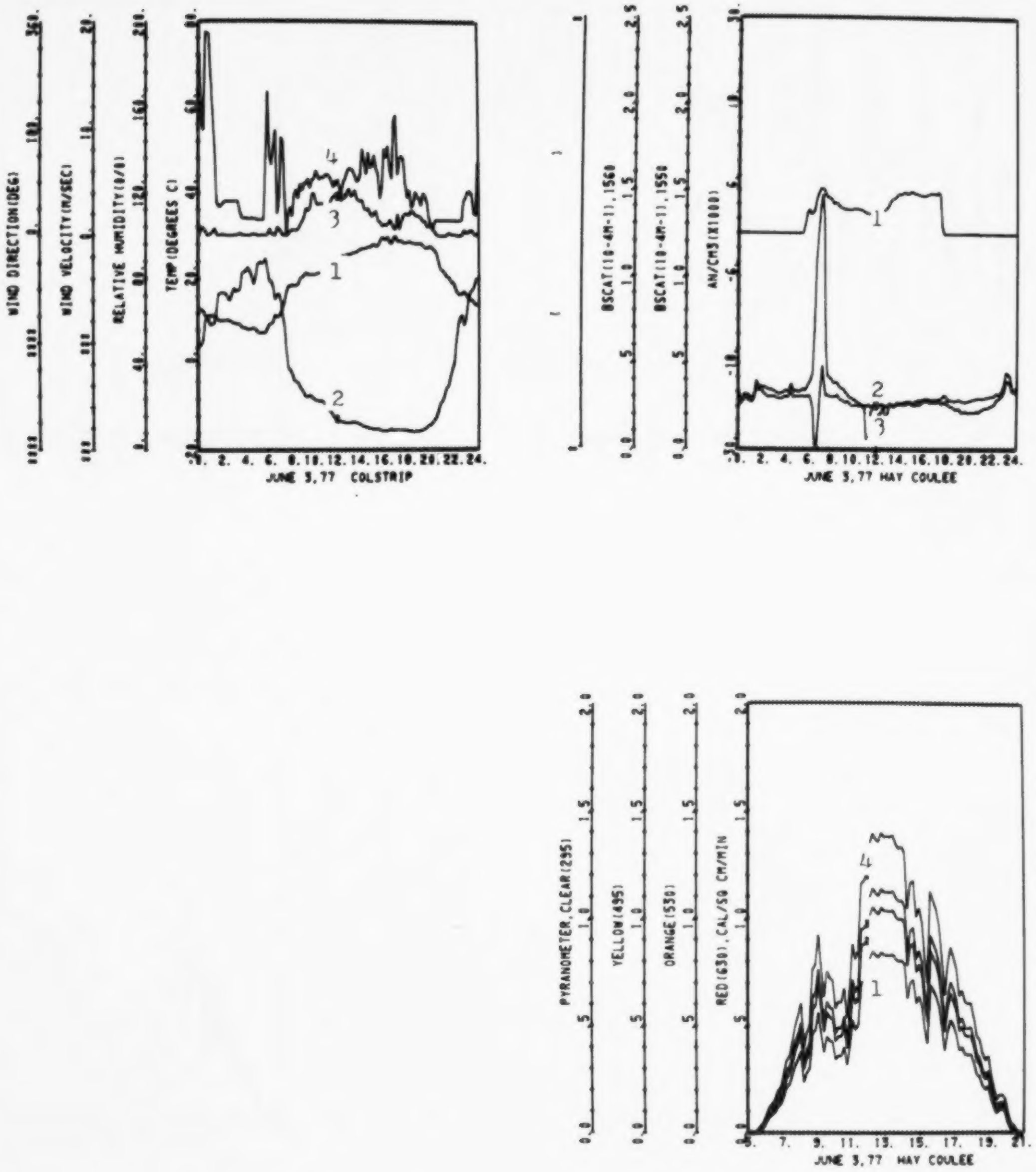


Figure 1.1b. Continued.

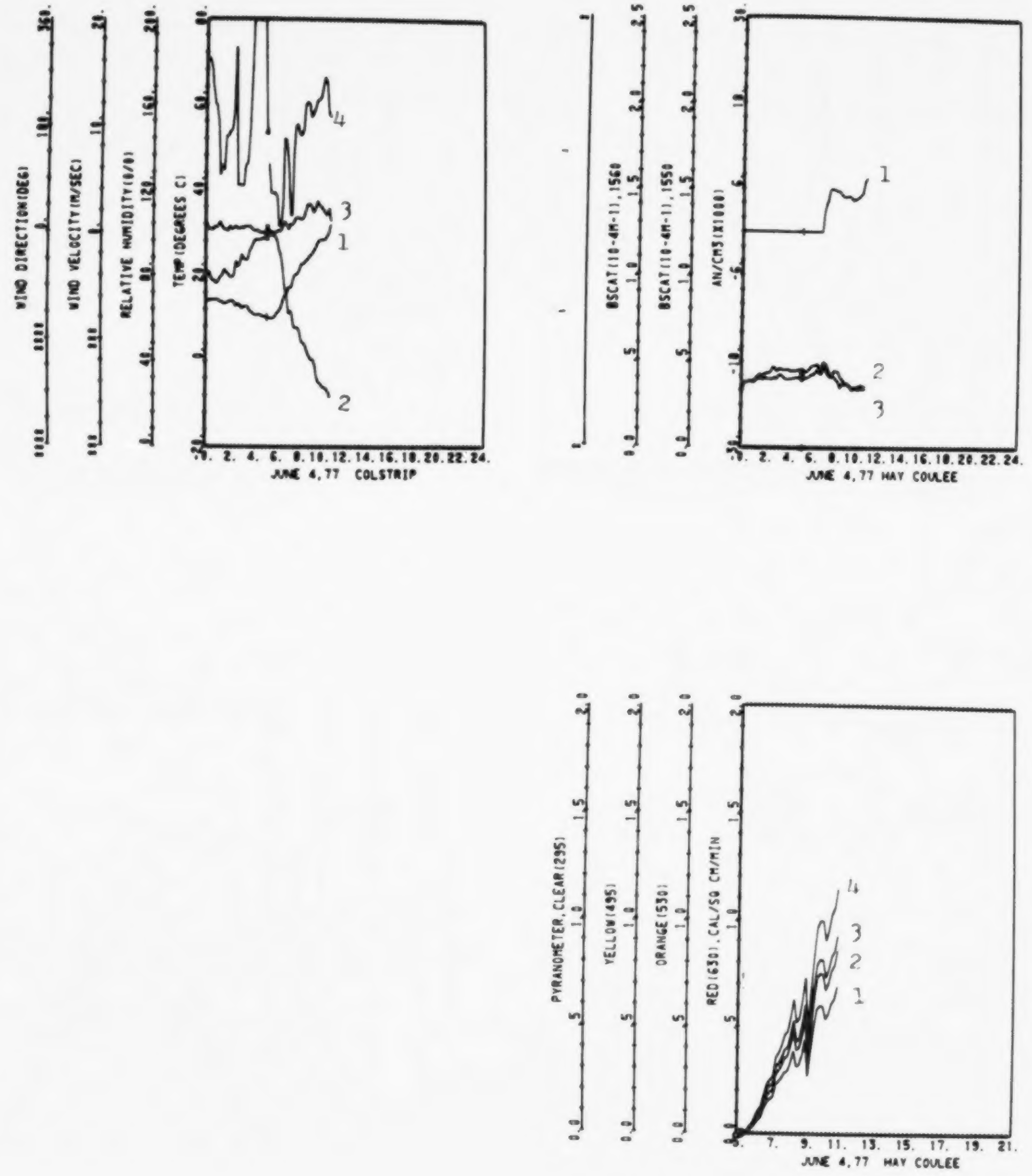
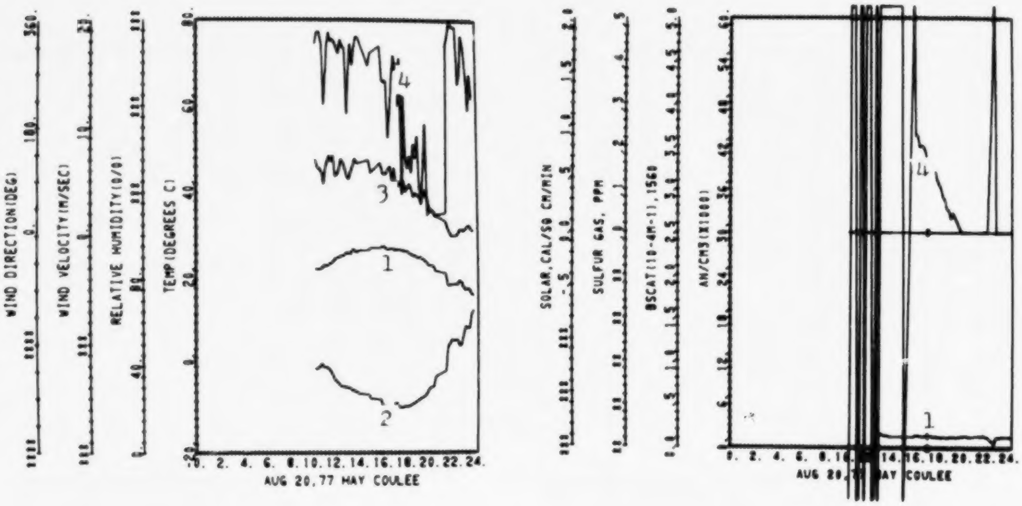


Figure 1.1b. Continued.



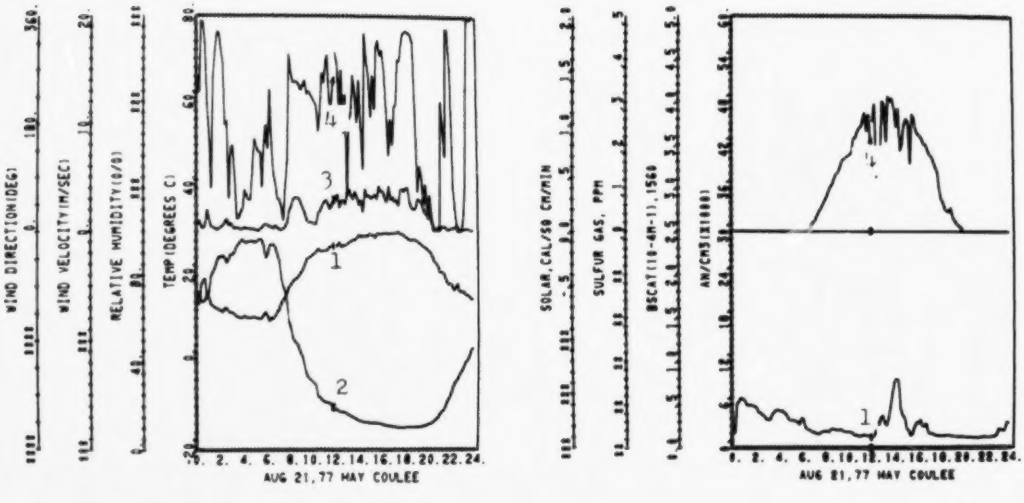


Figure 1.1c. Continuously measured parameters at Hay Coulee, August 20 - September 3, 1977. Reading from right to left, the vertical scales on the left of each graph refer to plots numbered 1, 2, 3, and 4 respectively. The horizontal scale refers to Mountain Daylight Time.

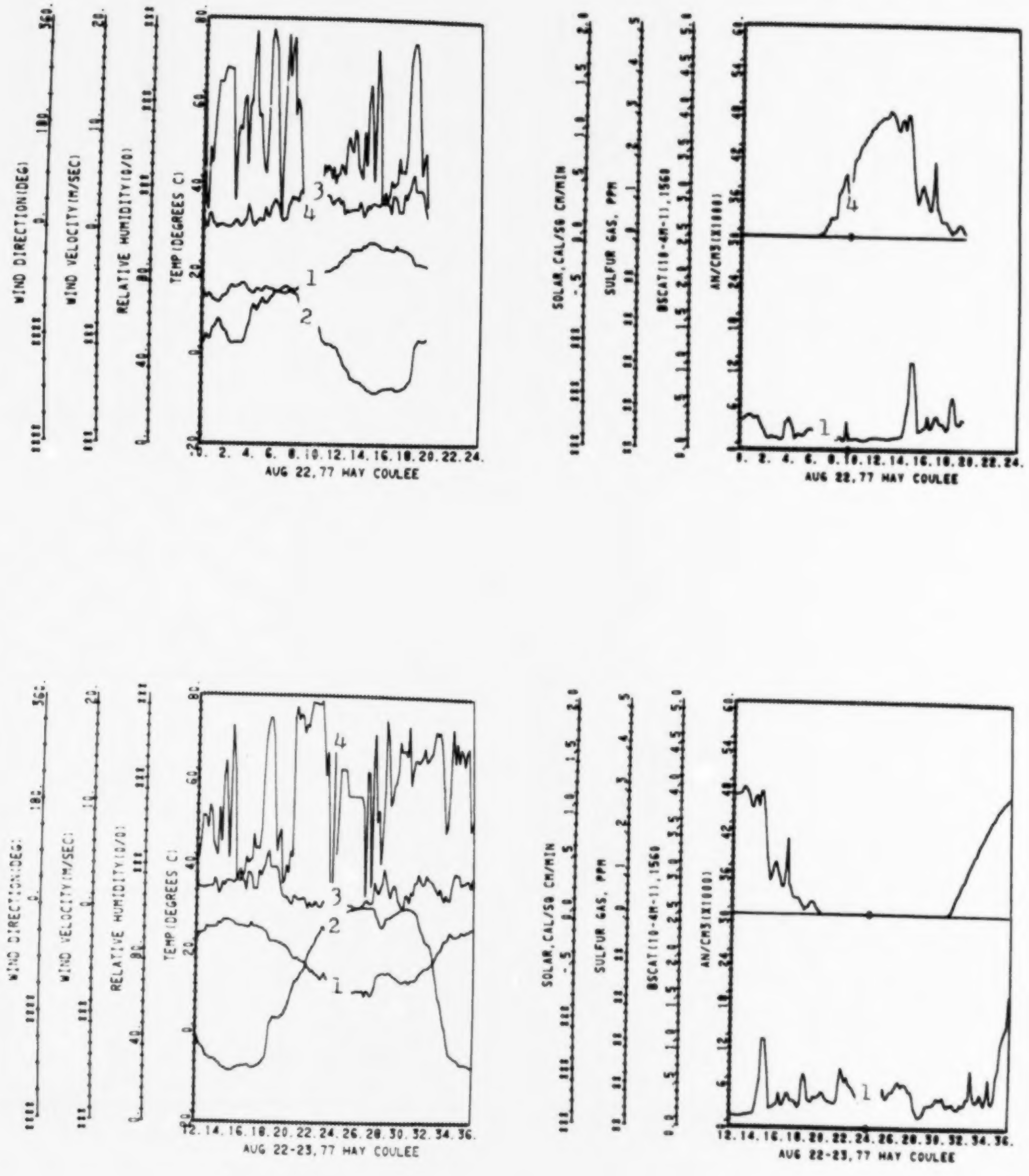


Figure 1.1c. Continued.

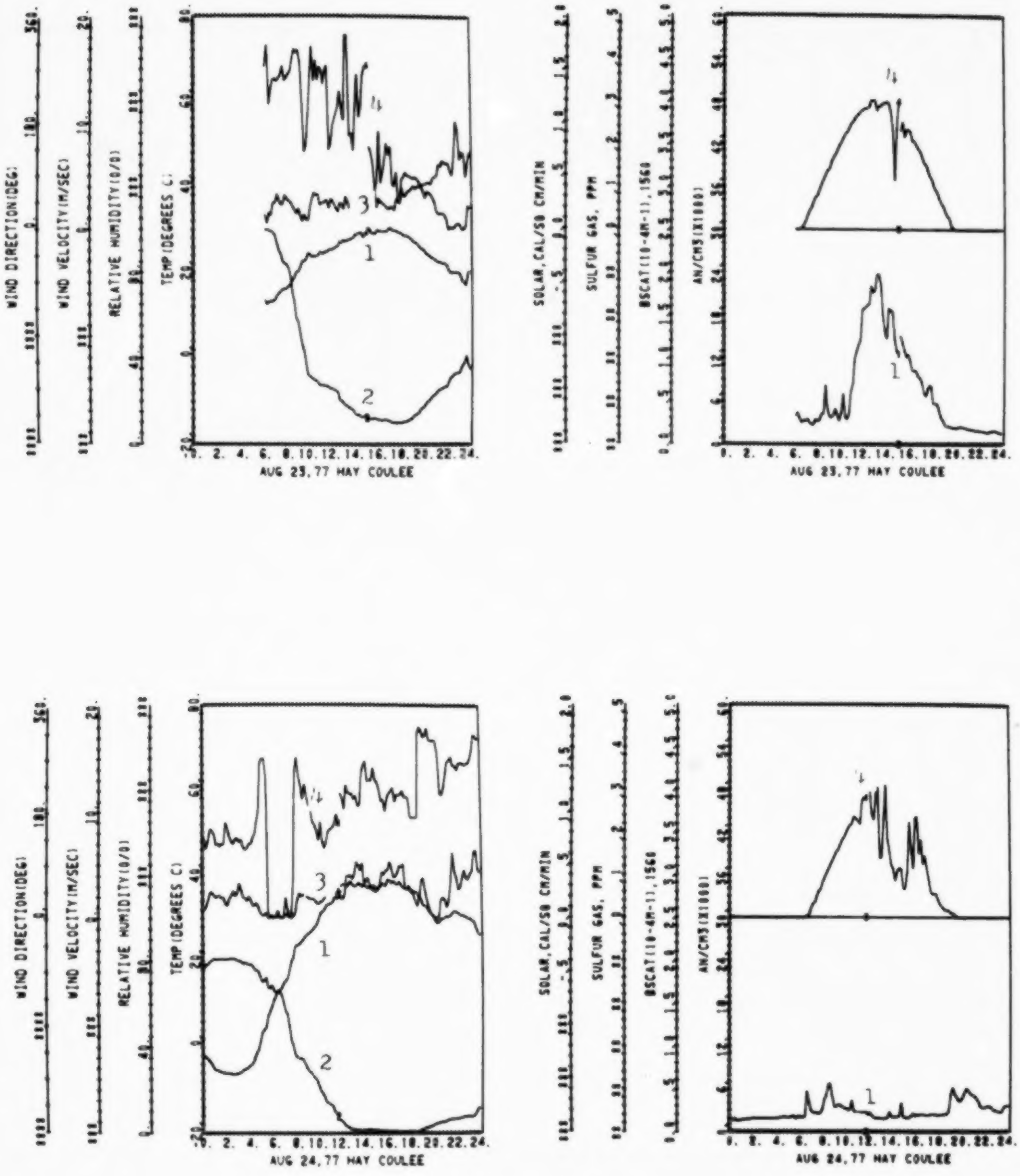


Figure 1.1c. Continued.

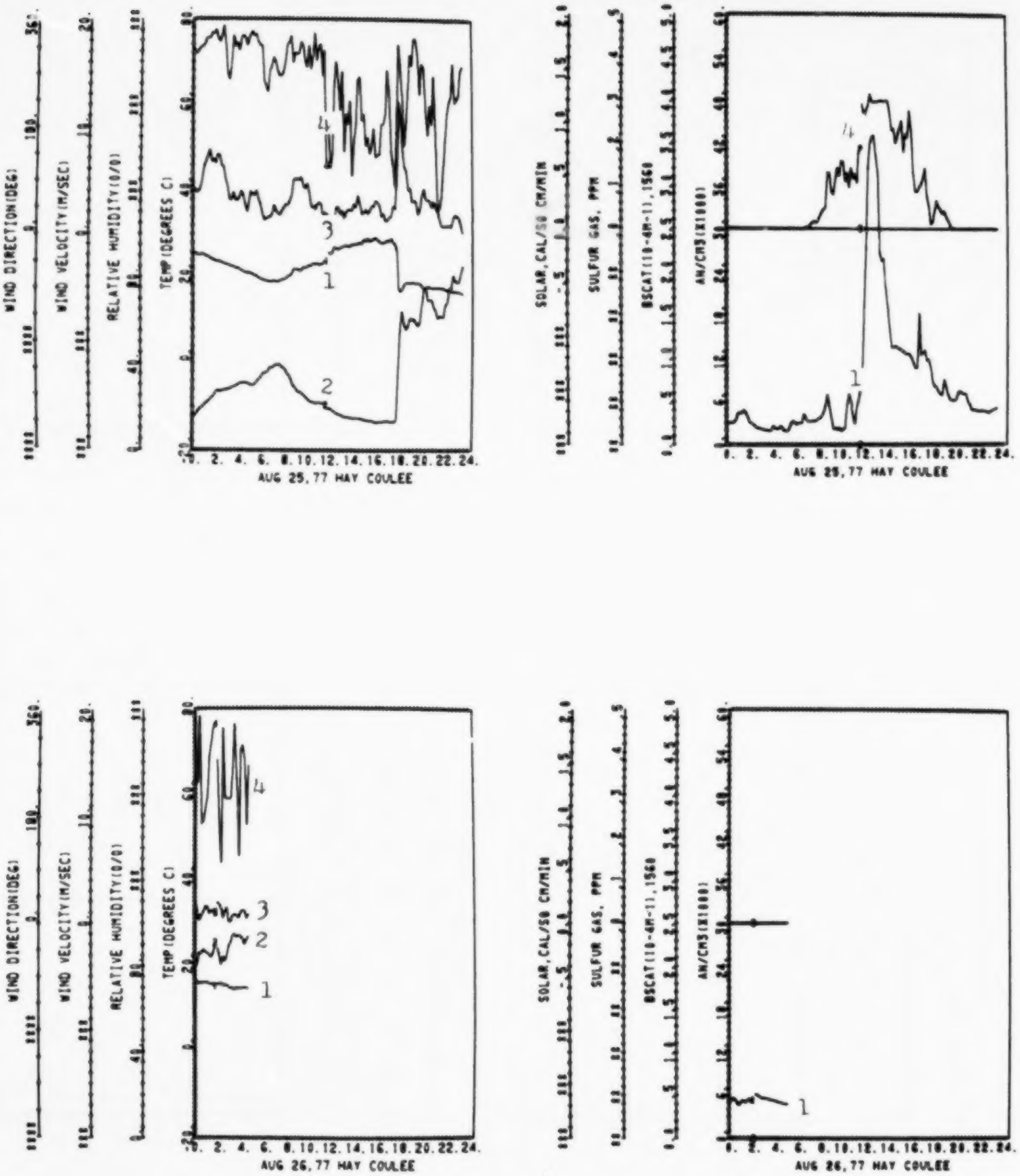


Figure 1.1c. Continued.

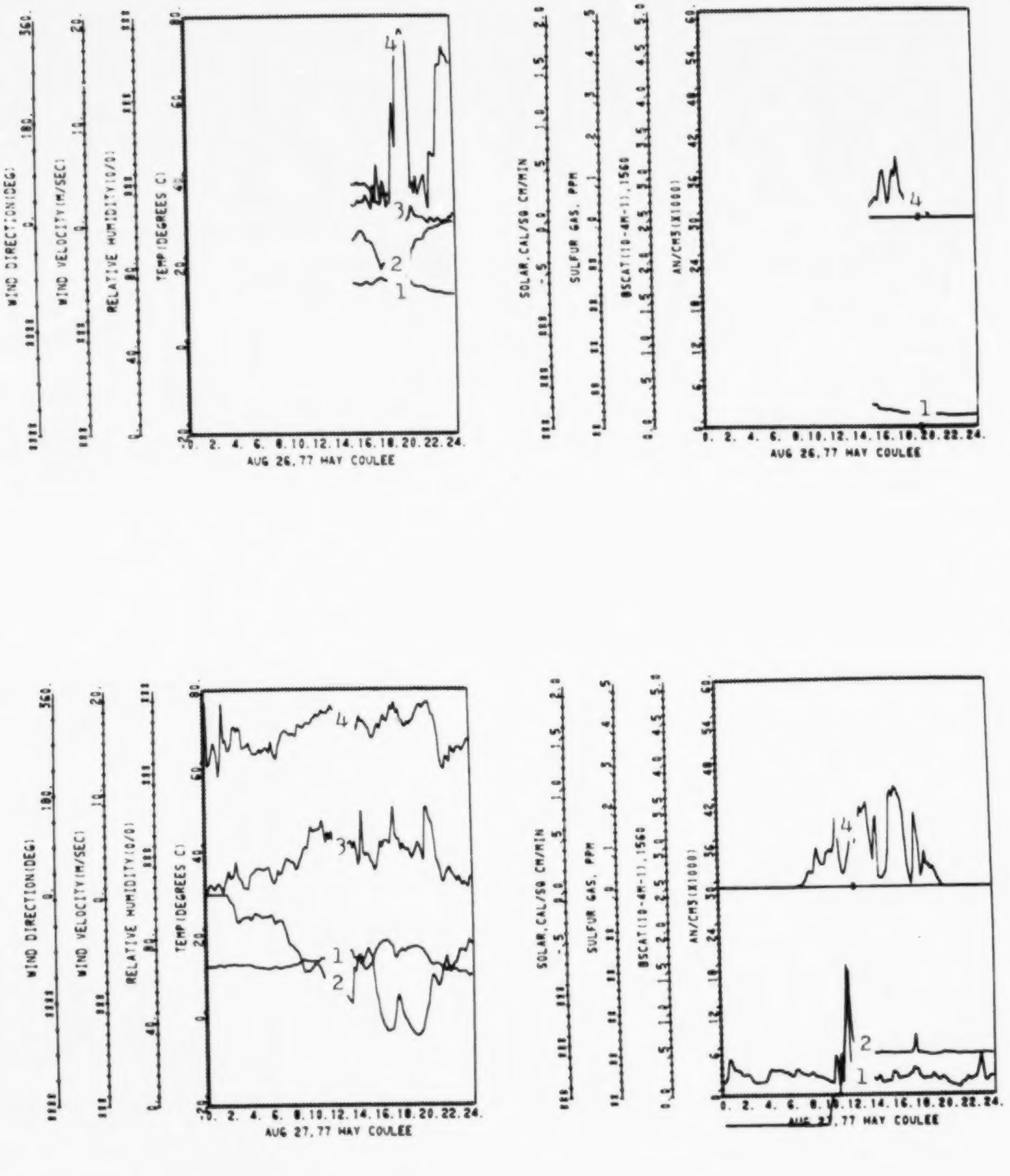


Figure 1.1c. Continued.

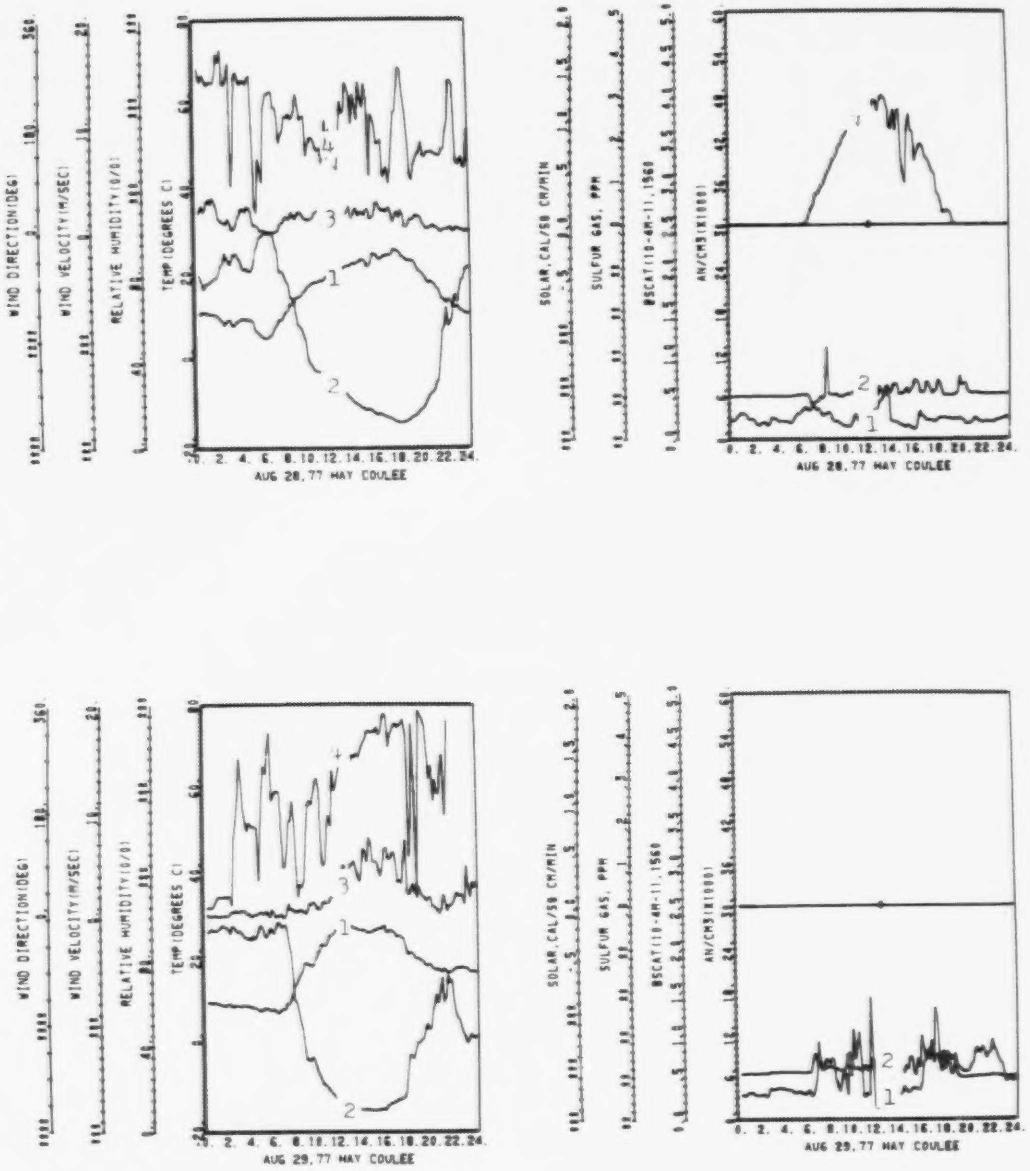


Figure 1.1c. Continued.

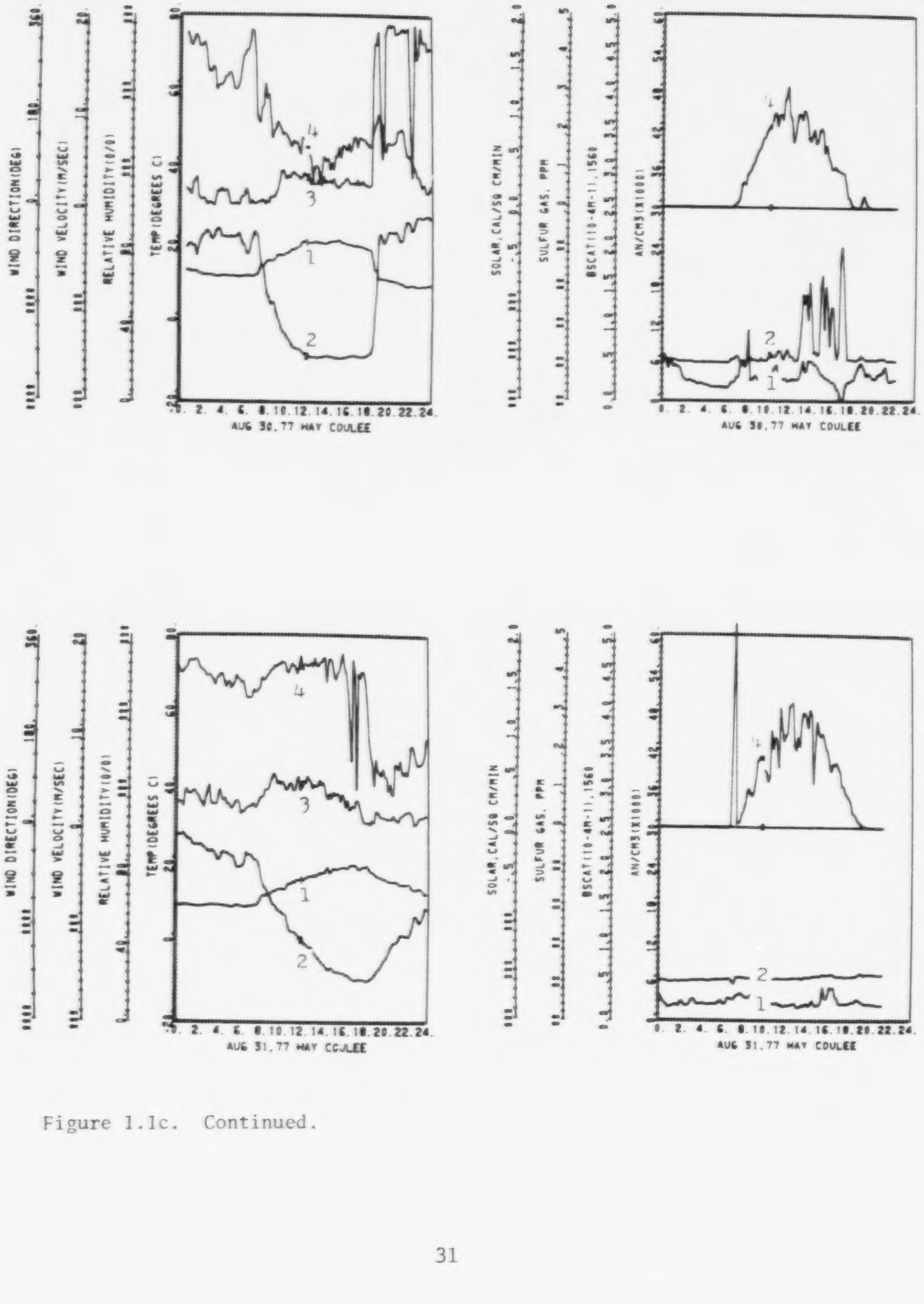


Figure 1.1c. Continued.

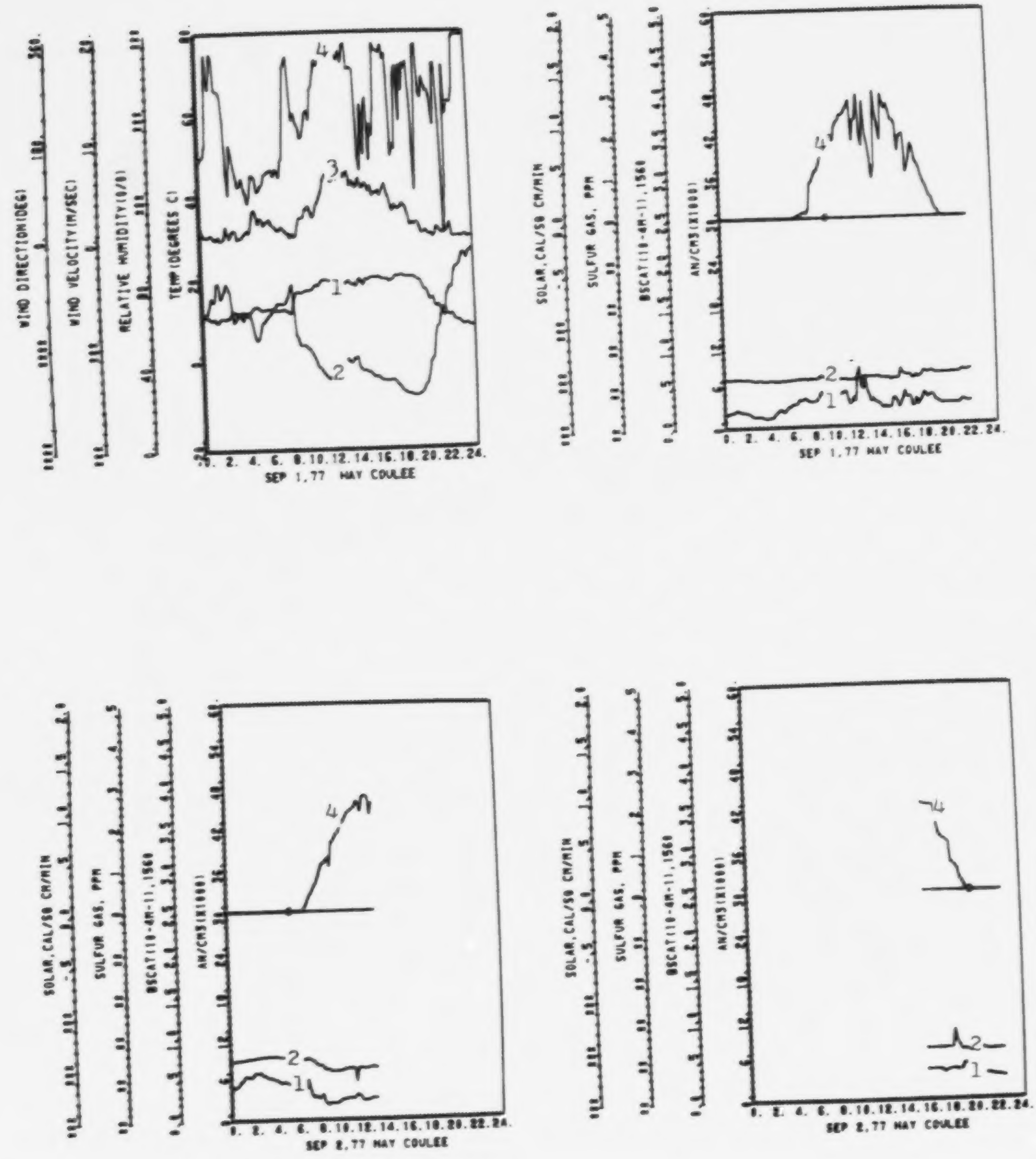
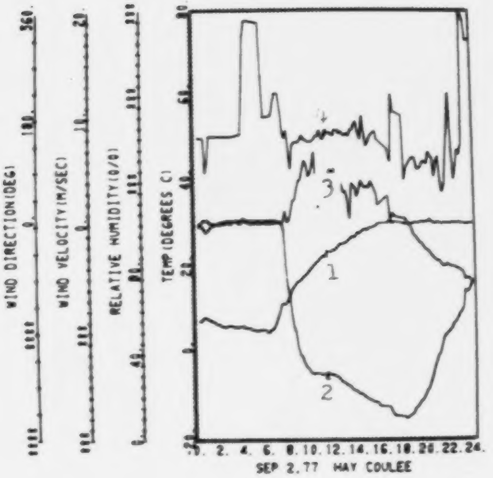
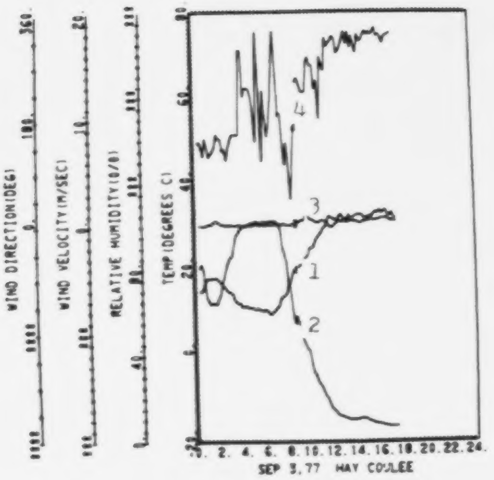


Figure 1.1c. Continued.





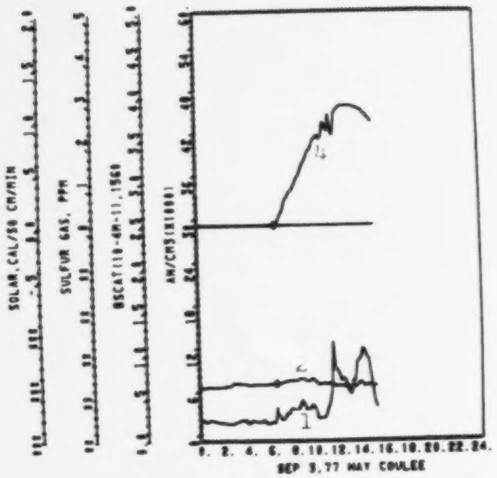


Figure 1.1c. Continued.

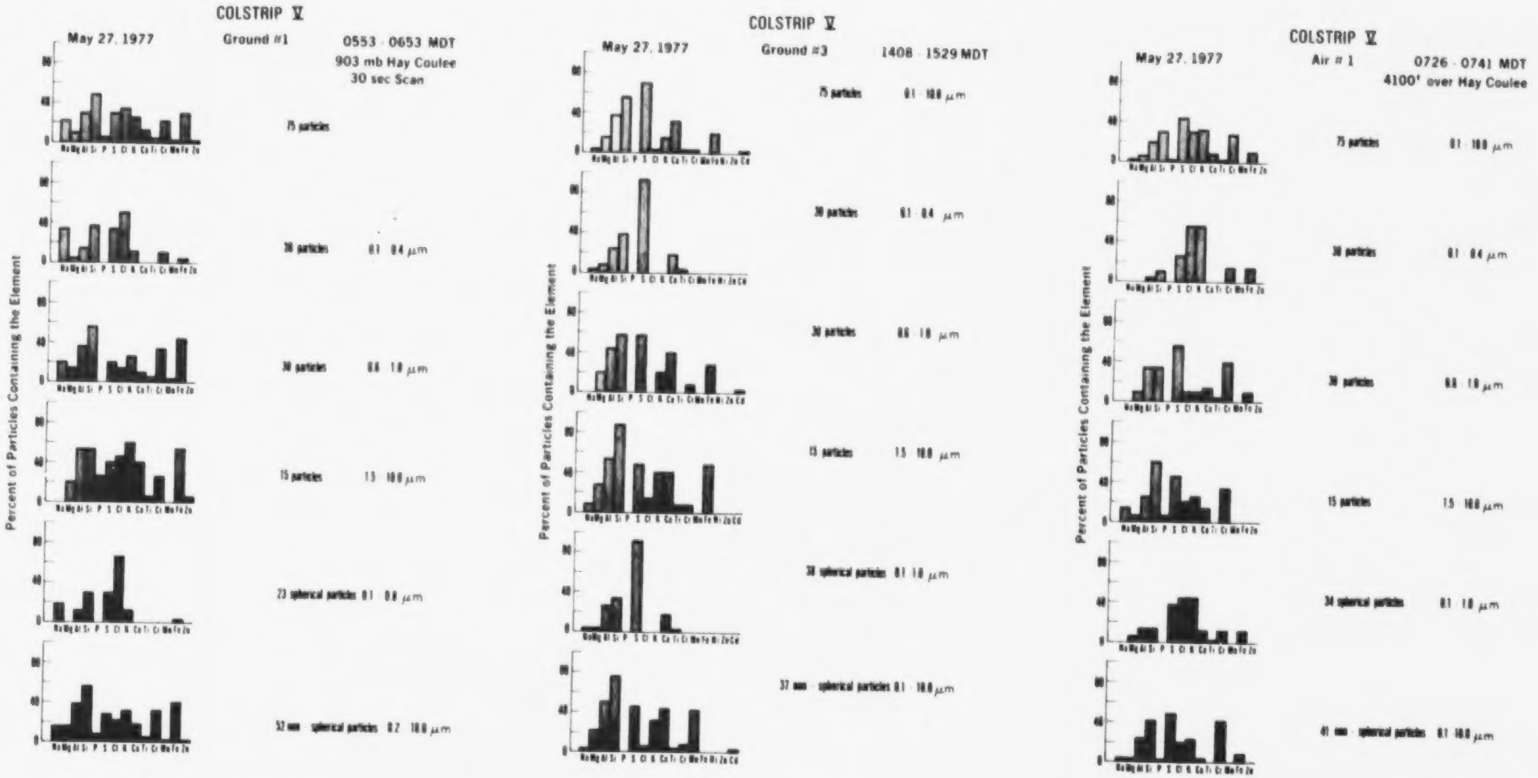


Figure 1.2. SEM-EDX derived classification of aerosol samples collected on membrane filters that were collected at Hay Coulee field site and with the aircraft over Hay Coulee on May 27, 1977. The top bar graph shows the elemental occurrence in the 75 particles that were examined. The next three graphs, reading downward, show elemental occurrence in the particles as classified according to particle diameter. The bottom two graphs show elemental occurrence in the particles as classified according to particle shape; the inclusive size ranges are shown for all particles in these two shape classifications.

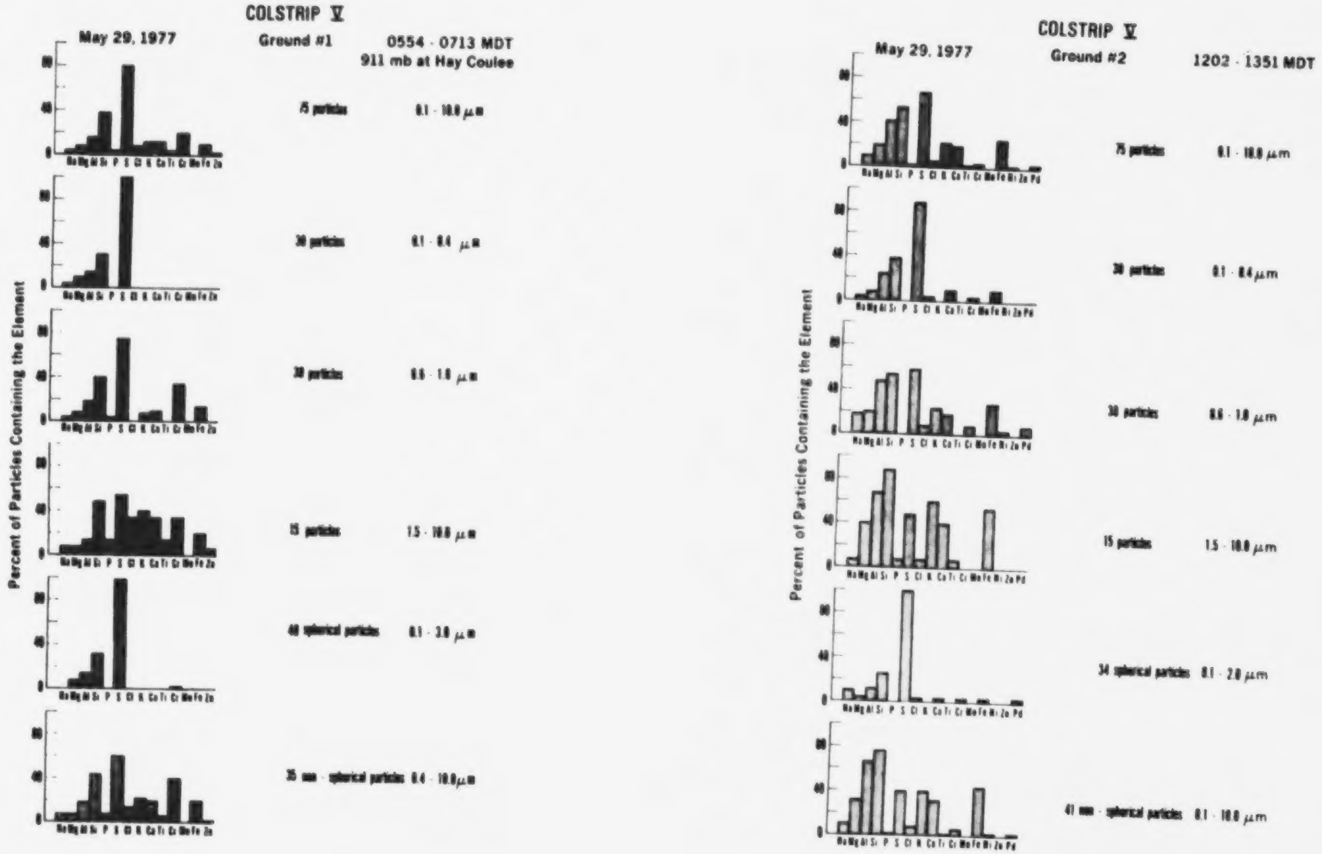


Figure 1.3. Same as for Figure 1.2, but, for samples collected May 29, 1977 at Hay Coulee field site (Ground #1 and #2), with the aircraft in the power plant plume near Colstrip, and with the aircraft over Hay Coulee, respectively.

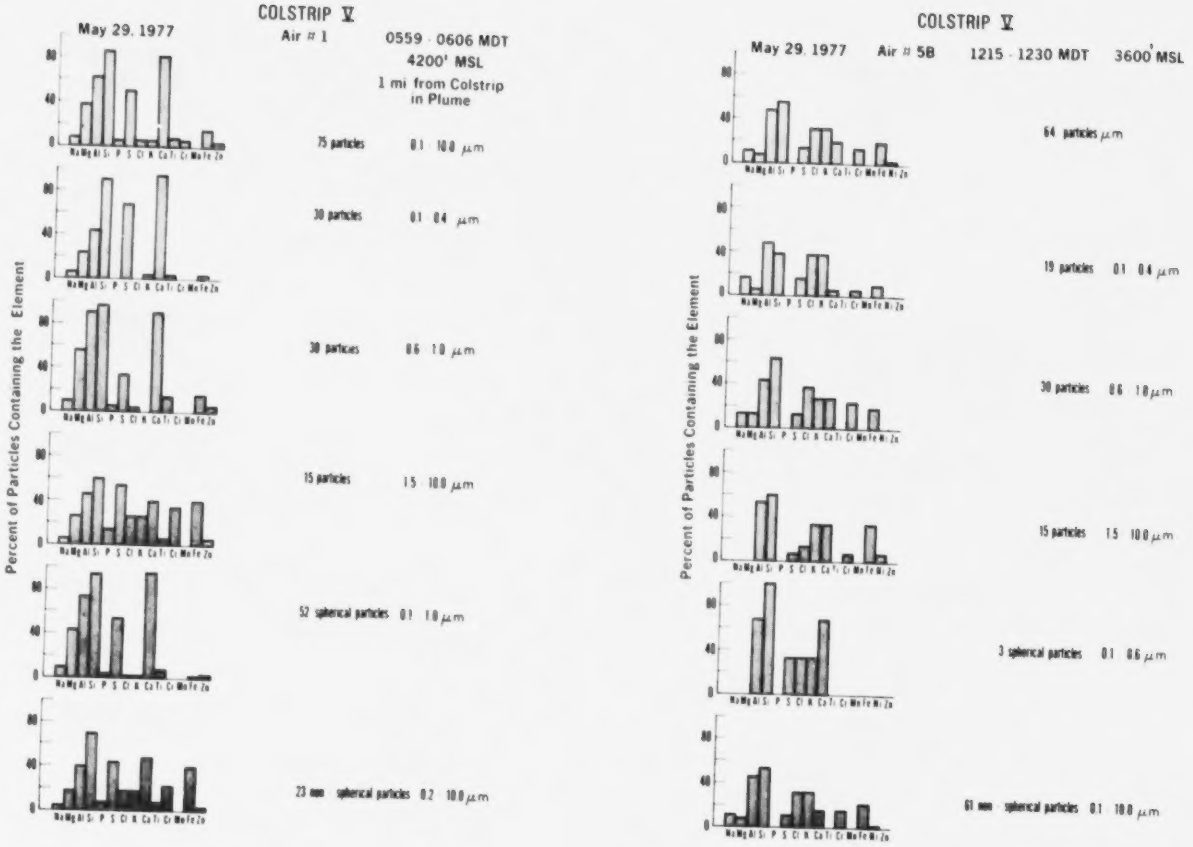


Figure 1.3. Continued.

evidence shows that there was no connection with the power plant plume? The unusually high incidence of Cl in the spherical particles is also puzzling, but may be indicative of some source other than the coal-burning power plant.

The May 27 Ground #3 sample was collected during the fumigation; spherical and irregularly-shaped particles appear in equal numbers. The elemental composition of the non-spherical particles is typical of natural aerosols collected at Hay Coulee. Almost all of the spherical particles contain S, in fact, some 60 percent contain nothing but S (considering detection only for Na and heavier elements). It is obvious in this sample, and apparent to a lesser degree in all the other examples shown here, that almost all of the spherical particles are found in the smallest size range.

Unexpected information was provided by SEM-EDX analysis of the May 29 samples. Both of the ground samples were almost identical to the Hay Coulee fumigation sample of May 27, i.e., approximately half of the particles were spherical, were in the smallest size range, and of these, on the order of 60 percent contained S. Subsequent examination of the instrumental record disclosed no obvious indication of plume fumigation, but the wind direction was northwesterly, although variable, and the AN concentration did exceed $1.2 \times 10^4 \text{cm}^{-3}$ in sporadic episodes during the day.

The May 29 Air #1 sample was collected in the plume about 1.6 kilometers from the power plant. Here, two-thirds of the particles were spherical, with a larger average diameter than for the spherical particles collected at greater distances from the power plant. Almost all the spherical particles contained both Si and Ca, with only about half containing S. The striking difference of elemental composition of spherical particles collected at 1.6 kilometers downwind as compared with that at a distance of 12 kilometers leads one to surmise that the particles that were in the plume at the shorter distance had all settled out by the time (one to three hours) that part of the plume reached Hay Coulee, and that these glassy mineral spheres had been replaced by H_2SO_4 or $(NH_4)_2SO_4$ particles that were the result of SO_2 oxidation, with subsequent gas-to-particle transformation. This hypothesis is supported by airborne measurements. Two examples can be cited at present:

(1) On August 27, 1977, between 0925 and 1035 MDT, measurements made in the plume (Figure 1.4) showed by far the greatest amount of light scattering at the shortest distance downwind. b_{scat} at greater distances was much less, with an indication of further decline with increasing distance. A less notable maximum followed by reduction of concentration with distance was measured for NO and NO_2. However, measurements of AN showed much higher (as much as an order of magnitude) concentrations at the greater downwind distances.

(2) On September 1, 1977, plume measurements were done to a distance of 50 kilometers downwind (near Ashland, Montana). AN concentrations were similar, at this distance, to concentrations measured at shorter distances, with the consequent requirement for the formation of particles to compensate for dilution as the plume expanded. We estimate the particle formation rate at 10^{11}sec^{-1} in the plume, on the basis of the AN concentration measurements and attempts to measure width and depth of the plume. Plume dimension

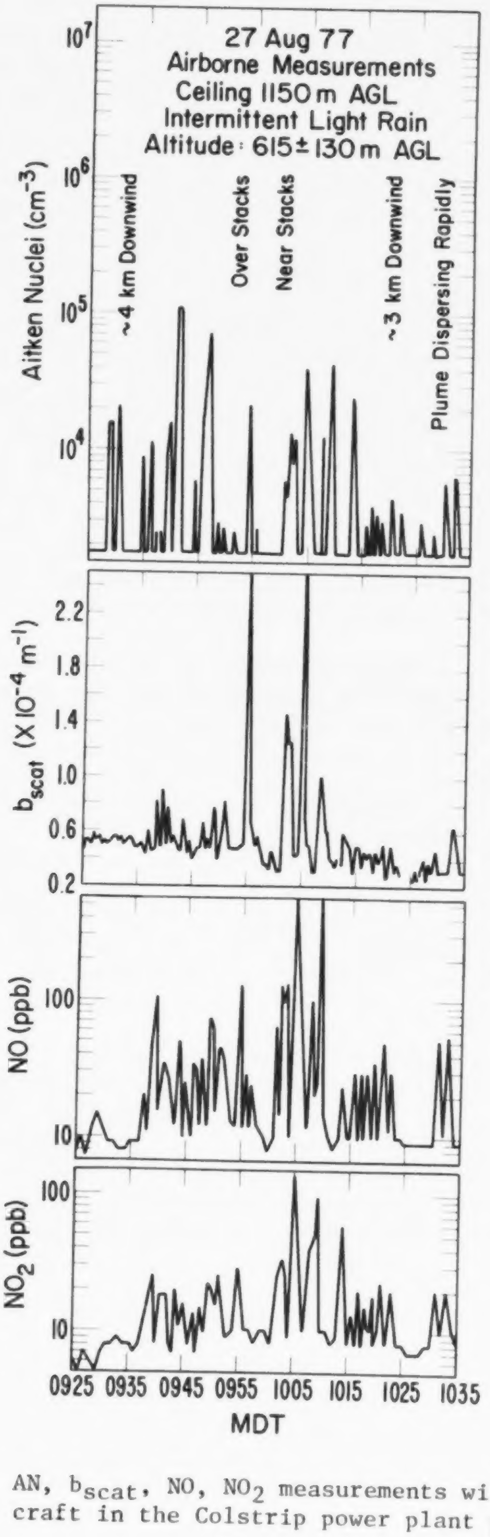


Figure 1.4. AN, b_{scat}, NO, NO_2 measurements with the aircraft in the Colstrip power plant plume.

measurements are very difficult at any distance greater than 15 kilometers because the plume becomes very patchy, and is also nearly invisible; it is detectable only by means of particle and gas measuring instruments. Hence, the imprecise nature of this estimate must be appreciated. This rate of particle formation is several orders of magnitude lower than that found in the smoke plume from the Four Corners power plant in northwest New Mexico (Pueschel and Van Valin, 1978).

The unexpected finding of plume particles in the Hay Coulee filter sample on May 29, 1977 prompted us to make a retrospective examination of the continuous AN concentration record of the last measurement period for evidence of plume exposure at Hay Coulee. Table 1.1 is a compilation of the pertinent information. We have listed all times when the AN concentration increased to at least double the level before and after. Episodes that, in our judgment, represented plume exposure are indicated by a "P" in the left-hand column. Any examples of steady AN concentration readings were interpreted as being due to natural causes not related to the plume; this kind of variation in AN concentration is common. Plume exposure was judged to have occurred when changes were large in comparison to the background, were abrupt, and were individually of short duration. On this basis, Hay Coulee seems to have experienced plume exposure 21 different times for a total of about 30 hours out of 340 hours of observation from 1300 MDT, August 20 to 1700 MDT, September 3, 1977. CCN and IN concentrations and b_{scat} are also listed in Table 1.1 whenever measurements were taken during an episode of elevated AN concentration; in addition, for comparison we have included many examples of CCN, IN, and b_{scat} levels when the AN concentrations were normal background. CCN levels seem not to be correlated with AN, but IN measurements with the continuously operating acoustic counter show IN concentrations to be five times or more above normal levels when plume parcels were present. The IN concentration by the filter method was much less correlated to plume presence; this method utilizes an integrated sample collected for one hour, and may be poorly suited to the short-lived episodes of exposure that occur at Hay Coulee. Also, these two methods of IN measurements respond to different mechanisms of ice crystal formation. While the predominant response to the acoustic counter is to contact nucleation, the nucleation and growth process of ice crystals on the filter is determined by the deposition or condensation-followed-by-freezing mechanisms. Which of these two mechanisms predominates on the filters depends upon the water solubility of the IN and upon the super-saturation with respect to water within the thermal diffusion chamber where the filters were developed.

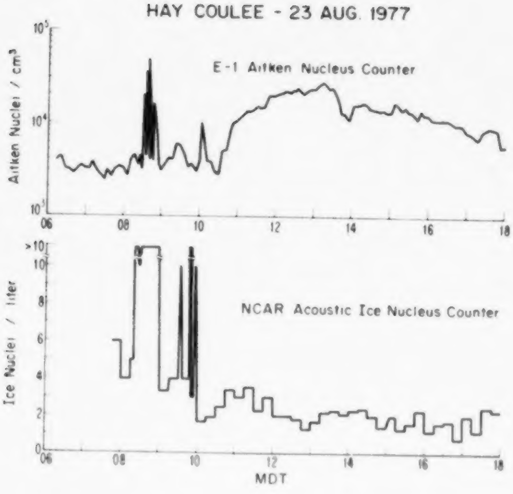
Figure 1.5a shows the record of AN and CCN concentrations at Hay Coulee on August 23, 1977, during an unmistakable plume strike from 0828 to 0845 MDT, a lesser exposure from about 1000 to 1010 MDT, and for the following time beginning at 1040 MDT, when there was no plume exposure, but the AN concentration reached a maximum of $2.8 \times 10^4 cm^{-3}$. During exposure, the AN concentration reached $4.8 \times 10^4 cm^{-3}$, the IN was offscale on the high side, and the patchy nature of the plume is indicated by the fact that, between pulses, the AN concentration fell almost to normal background levels. Figure 1.5b shows the record of AN measurements made with the instrumented aircraft

Table 1.1. AEROSOL PARAMETERS MEASURED AT HAY COULEE SITE, 20 AUGUST to 3 SEPTEMBER, 1977.

Date	Elevated AN Concen. Begin	Elevated AN Concen. End	AN Max. Concen. (X 10^3cm^{-3})	Character of AN Elevation	Wind Direction	Wind Velocity msec^{-1}	Other, Time of Measurements	AN Avg. Concen. (X 10^3cm^{-1})	b_{scat} (X 10^{-4}m^{-1})	CCN No. of Meas.	CCN Average (cm^{-3})	IN Filter Method No. of Meas.	IN Filter Method Concen. (ℓ^{-1})	IN Acoustic Concen. (ℓ^{-1})
P 8-20	2100	2400	6.8	Intermittent	calm									
8-21	0000	0630	7.8	Continuous, steady, gradually declining	calm									
8-21							0855-1025	2.2				1	3.1	
P 8-21	1200	1700	24.0	Continuous, lg. variation	N	3				5	602 ± 295	2	2.3	>10
8-21	2240	2400	4.1	Continuous, variable	calm									
8-22	0000	0200	7.3	Continuous, steady, declining gradually	calm									
8-22	0345	0430	5.0	Continuous, steady	calm									
8-22					NNE	4	0622-1110	1.9		4	472 ± 258	2	10.3	
P 8-22	1406	1515	20.0	Continuous, several pulses	N	3				4	577 ± 231	1	7.8	
P 8-22	2107	2230	10.0	Continuous, several pulses first	calm									
P 8-23	0828	0854	48.0	Many strong pulses	NW	2				2	1260	1	5.5	>10
P 8-23	1000	1010	10.0	Weak pulses	N	3								
8-23	1040	1900	28.0	Continuous, steady	N,NE	3				14	1130 ± 494	2	5.4	2.0
8-24	0800	0900	8.0	Gradual rise & fall	SW	2				2	525			2.0
8-24							1010-1155	2.6		4	578 ± 372			
8-24	1820	2120	7.8	Continuous, steady	NW	<2				4	385 ± 305	1	8.6	
8-25	0020	0120	5.1	Continuous, steady	NNW	7								
P 8-25	0636	0639	8.8	Weak pulses	WNW	3						1	1.6	
P 8-25	0810	0900	8.5	Continuous, variable	N	6				2	490			
P 8-25	1009	1047	10.0	Continuous, variable	NNW	4				2	460			
8-25	1058	1730	46.0	Continuous, variable, thunderstorm @ 1730	variable gusty					5	434 ± 264	2	6.6	4.0
8-26	0000	0940	8.0	Continuous, steady	lt & variable					3	1190	1	3.8	
8-26							1000-2400	2-3				1	2.7	
8-27							1235-1445	2.0	.6	6	338 ± 205			
P 8-27	1500	1530	4.4	Continuous, variable	WNW	3			.48	2	840			

Table 1.1. Continued.

Date	Elevated AN Concen. Begin	Elevated AN Concen. End	AN Max. Concen. (X $10^3 cm^{-3}$)	Character of AN Elevation	Wind Direc-tion	Wind Velo-city $msec^{-1}$	Other, Time of Measurements	AN Avg. Concen. (X $10^3 cm^{-1}$)	b_{scat} (X $10^{-4} m^{-1}$)	CCN No. of Meas.	CCN Average (cm^{-3})	IN Filter Method No. of Meas.	IN Filter Method Concen. (ℓ^{-1})	IN Acoustic Concen. (ℓ^{-1})
P 8-27	1653	1725	5.3	Continuous, variable	N	6(G18)			.48					
8-27							1800-2000	1.0	.47					
P 8-27	2230	2300	7.6	Continuous, variable	W	<2			.45					
8-28							0200-0600	1.5	.50					
8-28							0805-1145	2.8	.51	18	560 ± 206			
8-28	1310	1500	10.0	Continuous, steady	lt. & variable				.65	2	500	1	5.3	
8-28							1800-2400	2.0	.51					
8-29							1300-1600	3.0	.60	4	525 ± 185			
P 8-29	1605	1830	15.0	Continuous, variable	NNW	4			.65	6	1162 ± 379	1	3.5	10
8-29	1830	2300	10.0	Continuous, steady	lt. & variable				.52					
8-30							0200-0600	1.5	.50					
8-30							0825-1720	3.0	.55	14	535 ± 304			
P 8-30	1840	1905	8.0	Intermittent	NW	10			.55	2	1470			
P 8-30	2030	2120	6.5	Intermittent	NNW	8			.55					
8-31							0400-0600	1.8	.51					
8-31							0720-1445	2.6	.57	12	478 ± 191	2	10.4	
P 8-31	1530	1655	6.0	Intermittent	NW	3			.58	2	810			10
9-1							0200-0400	0.8	.55					
P 9-1	1000	1057	6.4	Continuous, variable	NW	4			.58	2	600			>10
P 9-1	1113	1234	11.1	Continuous, variable	NNW	6			.55	2	910			>10
P 9-1	1448	1830	6.3	Intermittent	NNW	4			.58	6	630 ± 153	1	4.6	>10
9-1	2200	2400	6.9	Continuous, steady	lt & variable				.65					
9-2	0000	0600	7.4	Continuous, steady, ground fog	calm				.75					
9-2							0720-1210	2-3	.60	2	770	2	4.7	
9-3							0000-0600	1.8	.62					
P 9-3	1043	1200	19.2	Continuous, variable	NNW	<2			.70			1	9.7	
P 9-3	1300	1445	15.0	Continuous, variable	NNW	<2			.65					



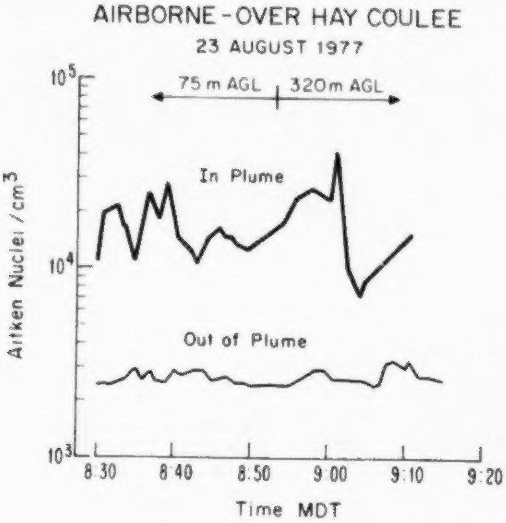


Figure 1.5a. (TOP) AN and IN concentrations measured at Hay Coulee showing power plant plume exposure from 0828 to 0854 MDT, and from 1000 to 1010 MDT.

Figure 1.5b. (BOTTOM) AN concentration measurements with the aircraft during the time of the Hay Coulee plume exposure. The elevation of the Hay Coulee site is 930 m MSL.

over Hay Coulee, both within and outside of the plume. These measurements are entirely consistent with, and supportive of, the measurements at the mobile laboratory in Hay Coulee.

Remote Sensing Laboratory

Measurements from Hay Coulee

The ground-based and airborne atmospheric characterization laboratories were supported by the remote sensing laboratory as described in the Third Interim Report (Abshire *et al.*, 1978). The laser radar (lidar) system measured optical backscatter coefficients throughout a hemispherical volume of three-kilometer radius. A complete set of measurements was recorded every hour on the hour; continuously whenever the aircraft was in the area; and at any other time there was an indication of plume activity. The effluent directly above the power plant was also probed from Hay Coulee, but the most interesting periods--before the daily breakup of the temperature inversion--were generally unavailable due to the intervention of a ridge into the line of sight between Hay Coulee and Colstrip. Average backscatter values within the lowest 500 meters are shown for each day in Table 1.2. Above 500 meters, backscatter values approaching clear air molecular scattering were usually obtained. Backscatter values frequently doubled in a series of short episodes indicating a plume strike or close approach, but there were seldom periods of prolonged high backscatter.

The lidar was usually operated in a dual polarization mode which allowed it to separate the backscatter of spherical particles, such as found in the power plant plume, from irregular particles, such as found in strip mine dust. The system was operated in a dual wavelength mode at 0.6943 μm and at 0.3472 μm, on September 3, 1977. Comparison on simultaneous backscatter values at the two wavelengths contains information regarding particulate size distribution, which is important in the determination of fallout rates. Reduction of the dual wavelength data was not completed in time to be included in this report.

The acoustic sounder was operated continuously during each observation period, providing a record of the existence and height of the temperature inversion. The usual pattern was observed with an inversion forming about midnight and breaking up during the mid-morning hours. Occasionally surface cooling from rain showers would cause the inversion layer to form before sundown and persist through the night into the next morning. The times during which a temperature inversion was detected are also shown in Table 1.2.

All of the solar photometry described in the Third Interim Report (Abshire *et al.*, 1978) was continued. In 1976, the following instruments were added:

(a) An infrared pyrheliometer with bandwidth 8-12 μm.

(b) An eight-channel photometer with channels each 0.01 μm wide and centered at 0.305, 0.317, 0.338, 0.382, 0.501, 0.875, 0.914, and 1.062 μm.

Table 1.2. SUMMARY OF 1977 REMOTE SENSING DATA

Day	Date	Average Daily Optical Backscatter in lowest 500 m ($m^{-1}ster^{-1}$)	Temperature Inversion Times		Location
			Morning Breakup	Evening Formation	
141	May 21	5.7×10^{-7}	0900 MST	2200 MST	Hay Coulee
142	May 22	5.6×10^{-7}	0830	2100	Hay Coulee
143	May 23	4.1×10^{-7}	1100	2300	Hay Coulee
144	May 24	5.8×10^{-7}	1000	2200	Hay Coulee
145	May 25	6.4×10^{-7}	1400	1830	Hay Coulee
146	May 26	4.0×10^{-7}	0900	1730	Hay Coulee
147	May 27	3.0×10^{-7}	0830	2200	Hay Coulee
149	May 29	3.8×10^{-7}	0900	2400	Hay Coulee
150	May 30	3.2×10^{-7}	1000	2200	Hay Coulee
151	May 31	3.2×10^{-7}	1030	2100	Hay Coulee
152	June 1	4.2×10^{-7}	1130	2230	Hay Coulee
153	June 2	6.4×10^{-7}	0930	2400	Hay Coulee
154	June 3	1.3×10^{-6}	0900	2300	Hay Coulee
155	June 4	4.2×10^{-7}	0930	None	Hay Coulee
232	Aug. 20	5.3×10^{-7}	0930	0100*	Hay Coulee
233	Aug. 21	6.6×10^{-7}	0900	2200	Hay Coulee
234	Aug. 22	6.4×10^{-7}	1100	1830	Hay Coulee
235	Aug. 23	5.2×10^{-7}	0900	2000	Hay Coulee
236	Aug. 24	4.7×10^{-7}	1030	0100*	Hay Coulee
239	Aug. 27	3.6×10^{-7}	0600	1700	Top of Butte
240	Aug. 28	3.3×10^{-7}	No Inversion		Top of Butte
241	Aug. 29	4.7×10^{-7}	No Inversion		Top of Butte
242	Aug. 30	3.2×10^{-7}	0900	2130	Top of Butte
243	Aug. 31	3.3×10^{-7}	No Inversion		Top of Butte
244	Sep. 1	3.6×10^{-7}	0930	0130*	Top of Butte
245	Sep. 2	4.2×10^{-7}	0930	2100	Top of Butte
246	Sep. 3	4.0×10^{-7}	1130	None	Top of Butte
247	Sep. 4	6.8×10^{-7}	No Data		Top of Butte

* Next Day

Figure 1.6 shows a plot of optical depth derived from the 8-12 μm infrared photometer. Knowledge of infrared optical depth, combined with measurements at visible wavelengths, contains information relating to scattering characteristics of clouds and aerosols and permits estimation of heat loss by infrared radiation from the ground.

The wavelength bands of the eight-channel photometer were chosen to provide data on the total water vapor in the sun-instrument path, (0.941 and 0.875 μm), total ozone content (0.305, 0.317, and 0.338 μm) and the size distribution of aerosol particles in the path (0.338, 0.382, 0.501, 0.875, and 1.062 μm).

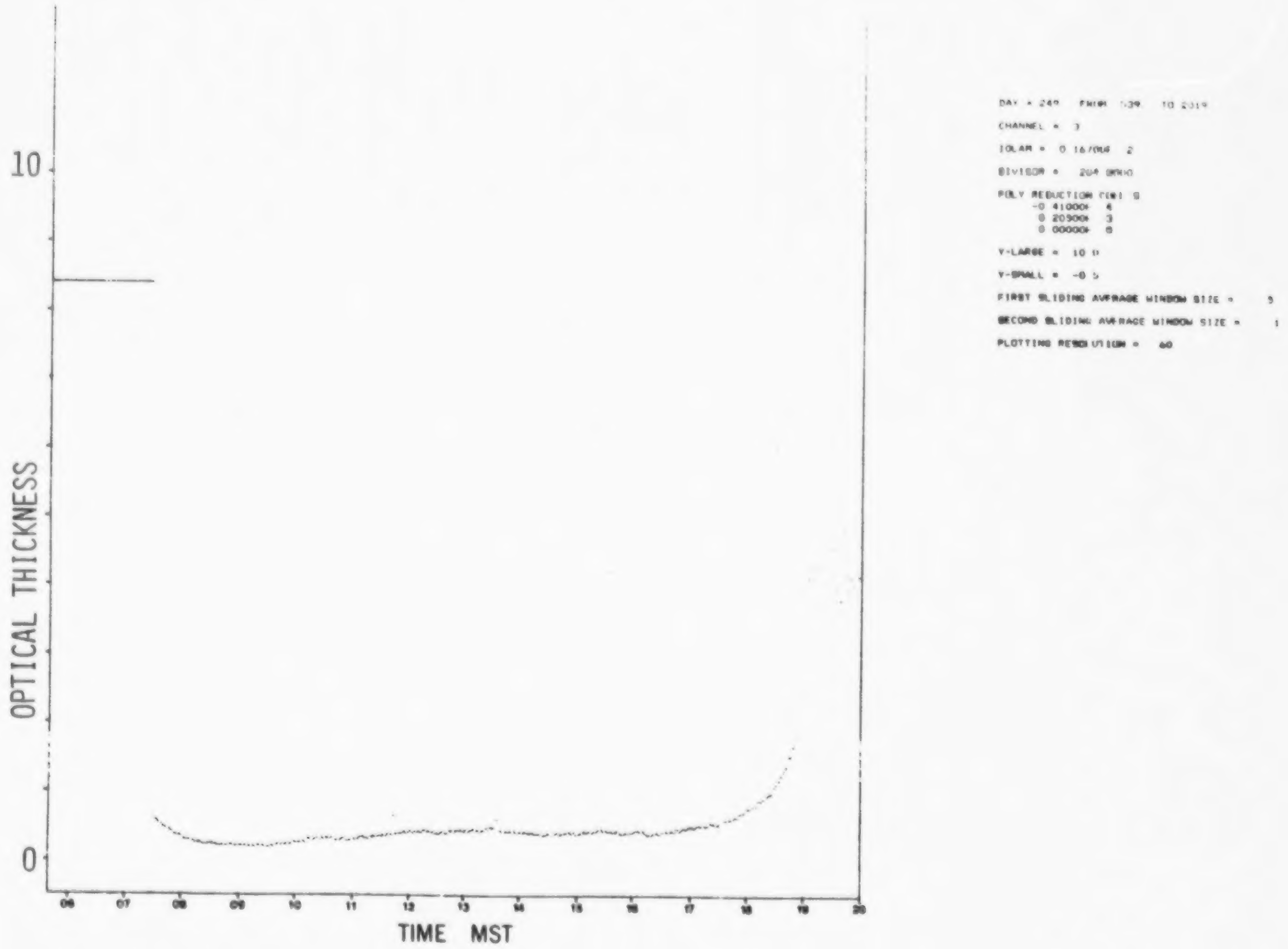
An instrument which measures the angular distribution of radiation at angles up to eight degrees from the sun was added in the spring of 1977. The shape of the intensity variation as a function of angular distance from the sun's center (solar aureole variation), varies with the size of scattering particles in the sun-instrument path. Thus, measurement of this angular variation can be used to deduce the size distribution of aerosols, including cloud particles for at least thin clouds. Figure 1.7 shows plots of the angular scattering function under a variety of conditions. The results obtained for the aerosol size distribution from the multi-wavelength photometer measurements are directly related to those obtained from solar aureole measurements.

Theory indicates that solar aureole measurement should work best to determine size distributions of larger particles (*e.g.*, >0.5 μm), while multi-wavelength photometric measurements may be the best method of determining size distributions of smaller particles (*e.g.*, <2 μm). The two methods are therefore likely to complement one another and should give results that are in agreement in the region of overlap.

Photographic records of sky conditions using wide-angle time-lapse photography have been obtained for each of the Colstrip experiments beginning with Spring 1976. This photographic record has proven extremely valuable in the determination of cloud types, geometry, and motions, or the presence of dust, smoke or any other conditions which might affect the observations.

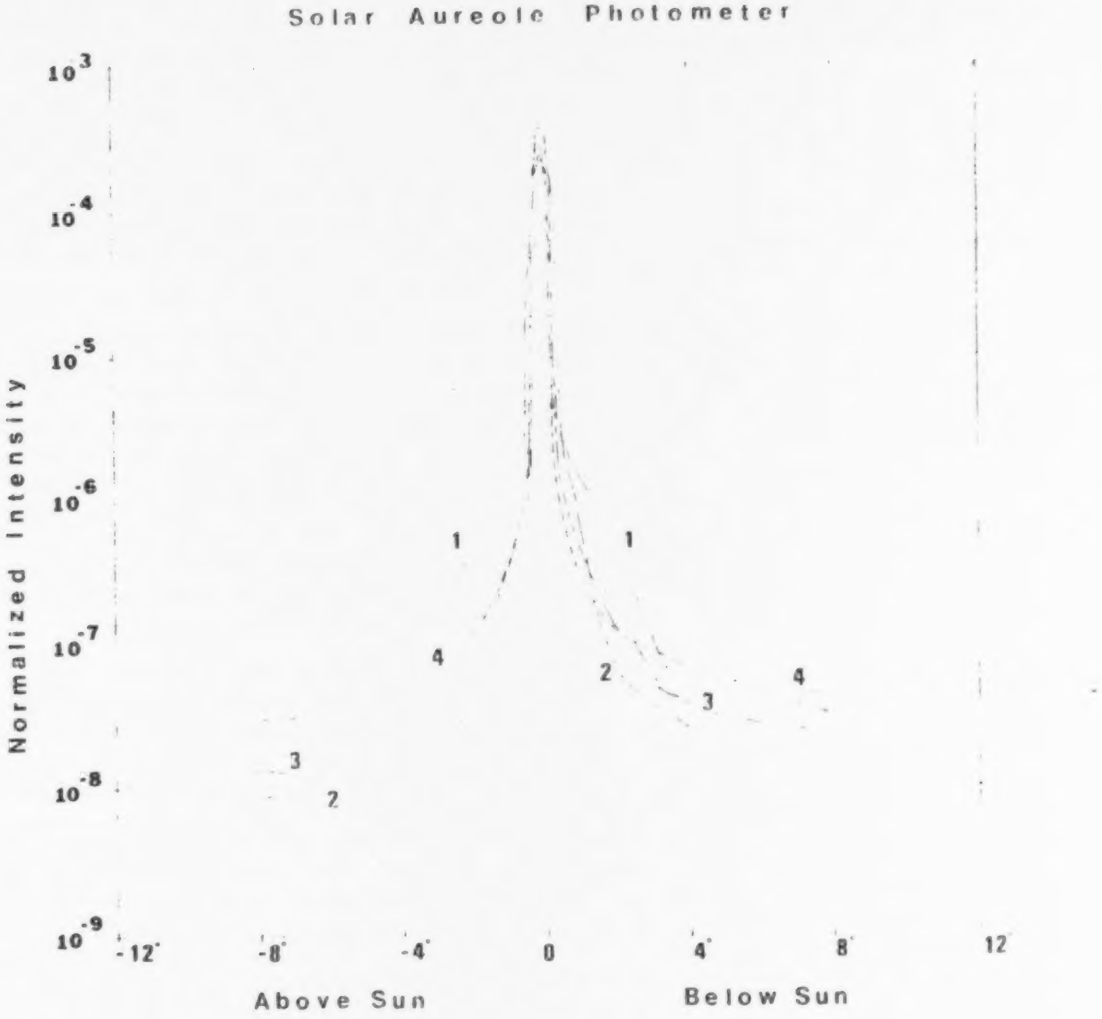
Clouds are mainly composed of larger particles, either water droplets or ice crystals. They generally affect the infrared radiation more than the visible wavelengths. Thin clouds, such as cirrus, and white broken cumulus do not result in significant reduction in total incoming radiation when averaged over time (say, an hour) because scattering and reflection compensate for the reduction in the direct component. A completely overcast sky with the sun's disc not discernible usually results in the incoming radiation being in the range of 15 percent to 25 percent for a clear day.

Only very preliminary analysis has been done on the 1977 spring and fall Colstrip solar photometry data. If these data are analyzed and compared to the 1975 and 1976 data, it should be possible to determine quite precisely the effects of the Colstrip power plant and the mining operations on the incoming solar spectrum. (Particular emphasis should be given to the 1977 fall data which were taken from the butte, nearer the power plant.)



```
DAY = 249   FROM 1:39   TO 20:19

CHANNEL =  3

IOLAM =  0 167064  2

DIVISOR =   204 0000

POLY REDUCTION COEF S
      -0 41000+  4
       0 20900+  3
       0 00000+  0

Y-LARGE =  10 0

Y-SMALL =  -0 5

FIRST SLIDING AVERAGE WINDOW SIZE =      5

SECOND SLIDING AVERAGE WINDOW SIZE =      1

PLOTTING RESOLUTION =   60
```

Figure 1.6. Optical depth derived from the infrared pyrheliometer.



Key:

1 —— Thin Stratus cloud-halo around sun with red boundary at 3° radius.

2 – – — Mostly clear sky with thin, turbulent wave cloud strands barely visible.

3 – – – Close-in aureole just visible due to very thin stratus clouds.

4 - - - - Aerosol haze seen out to several degrees.

Figure 1.7. Examples of solar aureole angular scattering functions.

Measurements from the Butte

For the second week of the fall 1977 period, the lidar system was moved from Hay Coulee to the top of a butte four kilometers southeast of the power plant (the Battelle No. 1 site). All of the previous remote sensing measurements were continued. In addition, from this vantage point, it was possible to track the plume as far as ten kilometers downwind each morning before the temperature inversion broke up. Data were recorded tracking both the plume and dust from the strip mines, using the polarization signatures of the two to separate them. Figure 1.8 is an example of the polarization signatures from both the plume and strip mine dust. This was recorded while helping the Battelle helicopter avoid the area of dust (which was invisible to the eye) while it was flying in and out of the power plant plume.

Two scan geometries were employed when tracking the plume from this site. In the "horizontal scan" mode, the lidar was scanned horizontally from the power plant downwind, with the elevation angle adjusted slightly to keep the system pointing at the center of the plume. This mode allowed the reconstruction of a horizontal backscatter profile and could be completed in roughly ten minutes. In the "vertical slice" mode, the system was scanned vertically up or down through the plume at a fixed azimuth angle, generating a vertical profile of the plume at that azimuth. The telescope was then moved five degrees and another vertical slice recorded, and so on. This technique required more time (approximately 45 minutes) to complete a scan, but produced vertical, as well as horizontal, information.

Since these were new types of information for us, the computer data reduction programs required had not been written and are still being developed. Some results of these scans have emerged and, although preliminary in nature, are very informative. Figure 1.9 shows a data set using the vertical slice mode. At the top is a plan view showing the relative positions of several vertical slices. The backscatter profiles of each vertical slice were projected onto a plane at an azimuth of 30 degrees, roughly normal to the prevailing wind. It is these profiles which are displayed in the lower portion of the figure. The range of backscatter coefficients in each profile is subdivided into thirds by the profile contours. Each profile is constructed to the same scale, with the vertical being magnified five times relative to the horizontal to bring out more vertical detail. This data set was recorded at 0730, September 3, 1977, with the plume trapped in stable air under a temperature inversion. The horizontal diffusion of the plume as it proceeds downwind is apparent. The 330-degree and 340-degree profiles suggest the separation of a section from the lower portion of the plume and the fallout of heavier particles. This is being investigated through the dual wavelength backscatter coefficients. The last four, especially the 80-degree profile, show the plume to be non-uniform in sections, even six kilometers downwind from the stack. This propagation of the plume in relatively small, but dense parcels support similar observations from the atmospheric characterization aircraft and ground station.

This highly preliminary analysis of the plume tracking data provides convincing evidence of the importance of the technique to understanding diffusion processes under the stable boundary layer.

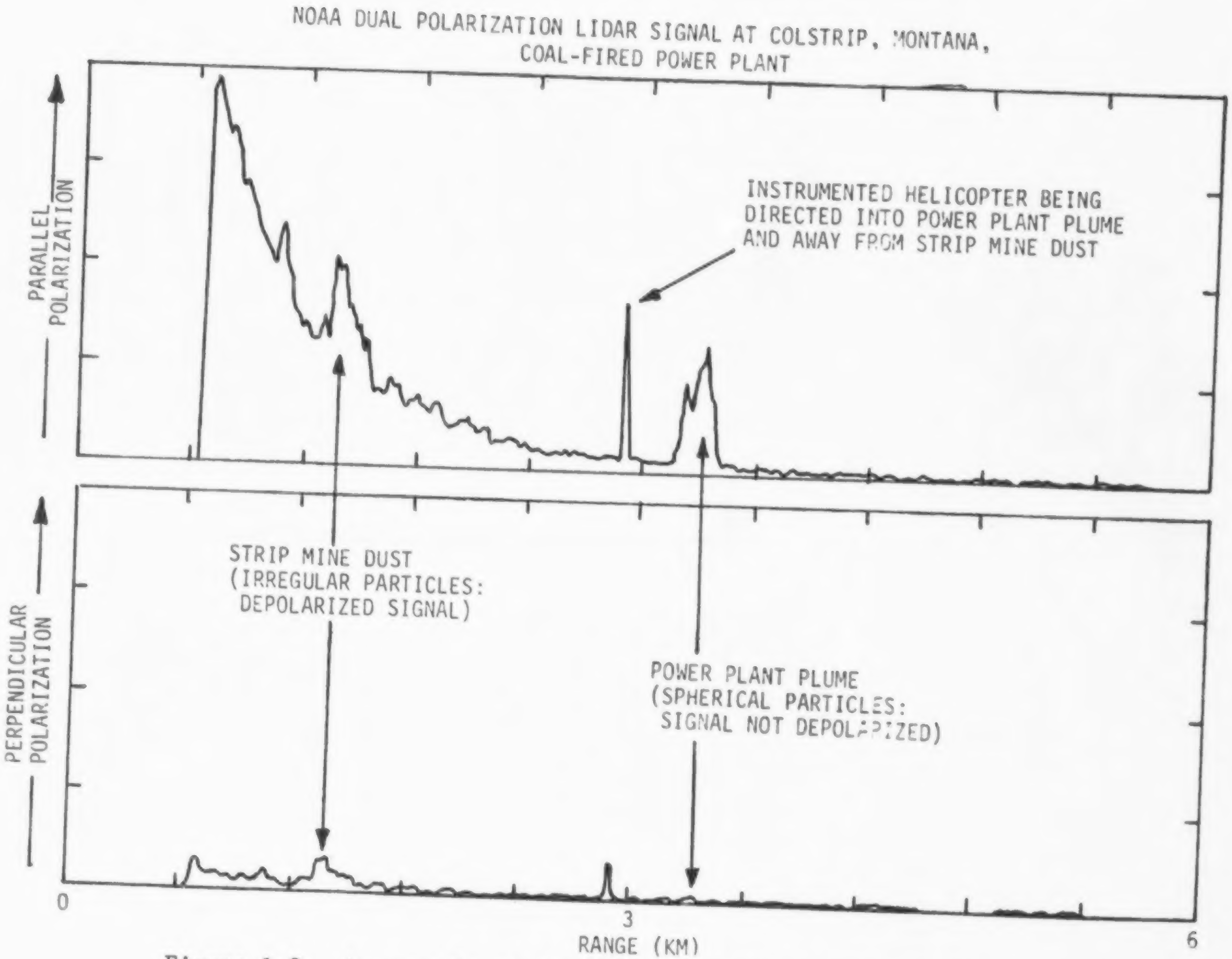


Figure 1.8. An example of lidar polarization signatures showing strip mine dust, power plant plume and the Batelle helicopter.

NOAA LIDAR OPTICAL BACKSCATTER CONTOURS
OF THE COLSTRIP, MONTANA, POWER PLANT PLUME, SEPT. 3, 1977.

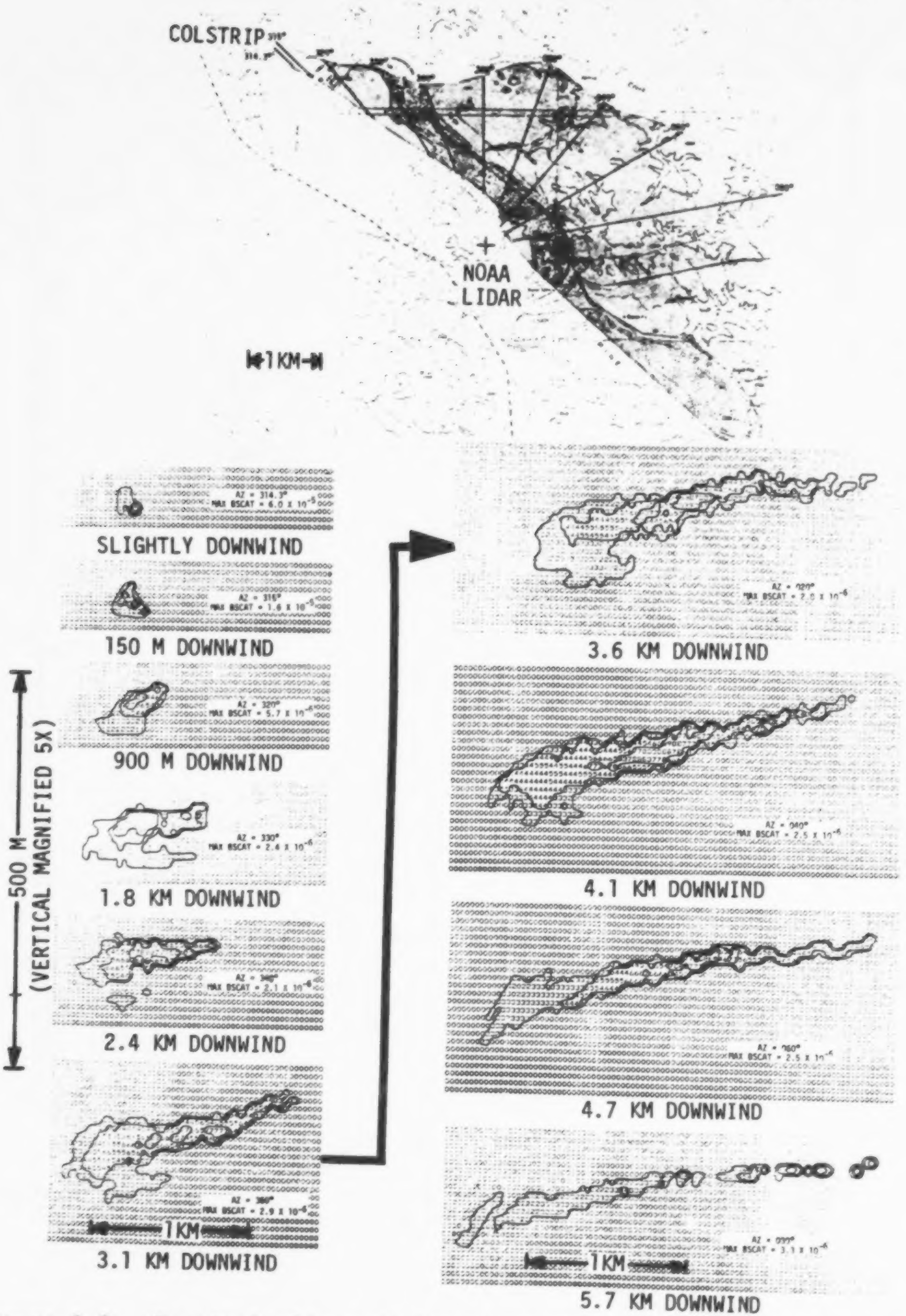


Figure 1.9. Vertical slices of the plume obtained by the lidar. The vertical and horizontal scales shown apply to all of the contour plots. The vertical is expanded 5X to bring out the complex structure.

CONCLUSIONS

The most important findings of this report are as follows:

(1) The aerosol in the vicinity of Hay Coulee may be classified into two main types of particles; (a) a man-made aerosol that consists of small spherical particles (dominating the 0.1 μm < D < 0.4 μm size range, *i.e.*, the smallest size range included in this study) composed mostly of S (plus O, N, or H) or of Cl compounds, and (b) a natural aerosol consisting of non-spherical particles that are mostly alumino-silicates with significant occurrences of the metallic elements, and which occur most frequently in the 0.6 - 1.0 μm diameter size range.

(2) Correlation of the aerosol measurements with existing meteorological conditions suggests that the Colstrip power plant is a sufficient, but not exclusive, condition for the existence of a man-made aerosol at Hay Coulee.

(3) Two types of particles exist in the power plant plume. At short distances from the plant, the particles are relatively large (optically significant), glassy, alumino-silicate spheres, with significant incidence of Ca and S. At large distances the sub-optical S-containing aerosol predominates; this small aerosol is apparently the result of a gas-to-particle conversion process.

(4) During the last observational period (20 August to 3 September 1977), Hay Coulee was exposed to plume parcels for about ten percent of the time. During the exposure periods, the concentration of pollutants varied abruptly and frequently approached background levels. These exposures result in the dry deposition of gases and aerosol that is potentially significant to the ecosystem.

(5) The power plant effluents increase the IN concentration, as measured by the acoustic IN counter, by an order of magnitude. This has possible consequences in the formation and distribution of precipitation.

(6) The plume, as tracked by the lidar, shows evidence of breaking up into inhomogeneous parcels within six kilometers from its source, even in a stable atmosphere. This is supported by the sporadic nature of each plume strike at Hay Coulee and by the in-plume measurements of the aircraft.

(7) The lidar, operating from a suitable vantage point and in conjunction with an aircraft equipped to measure particle size distributions, can probe the volume of the plume in high spatial resolution up to ten kilometers

from its source when inversion trapping is dominant. Such data can lead to an understanding of site-specific diffusion processes, the identification of local "hot-spots", and the construction of particle fallout maps.

REFERENCES

Abshire, N. L., V. E. Derr, G. T. McNice, R. Pueschel, and C. C. Van Valin. 1978. Integrated Aerosol Characterization Monitoring, Colstrip, Montana. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Third Interim Report, Colstrip, Montana, E. M. Preston and R. A. Lewis, eds., EPA-600/3-78/021, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 291-321.

Parungo, F. P., E. Ackerman, R. H. Proulx, and R. F. Pueschel. 1978. Nucleation Properties of Fly Ash in a Coal-Fired Power Plant Plume. Atmospheric Environment, to be published.

Pueschel, R. F., and C. C. Van Valin. 1978. Cloud Nucleus Formation in a Power Plant Plume. Atmospheric Environment, in press.

Pueschel, R. F., C. C. Van Valin, and F. P. Parungo. 1974. Effects of Air Pollutants on Cloud Nucleation. Geophysical Research Letters, 1(1). pp. 51-54.

Schnell, R. C., C. C. Van Valin, and R. F. Pueschel. 1976. Atmospheric Ice Nuclei: No Detectable Effects from a Coal-Fired Power Plant Plume. Geophysical Research Letters, 3(11). pp. 657-660.

Van Valin, C. C., R. F. Pueschel, F. P. Parungo, and R. H. Proulx. 1976. Cloud and Ice Nuclei from Human Activities. Atmospheric Environment, 10(1). pp. 27-31.

SECTION 2

BASELINE CHARACTERISTICS OF PRODUCER AND INVERTEBRATE POPULATIONS AND CERTAIN ABIOTIC PARAMETERS IN THE COLSTRIP VICINITY

J. L. Dodd, J. W. Leetham, T. J. McNary,
W. K. Lauenroth and G. L. Thor

ABSTRACT

Presented in this section is a culmination of the various facets of the studies conducted on the four field sites near the Colstrip power plant. A majority of the data has been reported in previous interim reports and is not repeated here. Presented here are some physical and chemical properties of the soils of the four sites; some recently developed data on herbage and litter dynamics and plant phenology, the chemical composition of the major plant species and their variation among sites, and finally a summarization of the arthropod population censusing done in 1974 and 1975. The principal objective of this portion of the overall coal-fired power plant project was to establish a set of baseline data prior to the introduction of air pollutants from the Colstrip power plant. The data presented here and in previous reports provide ecosystem level baseline data on the four field sites in hopes that future comparisons can be made after extended periods of pollutant exposure. A majority of the data indicates a good homogeneity among the four field plots with occasional variation in specific components such as certain plant or arthropod species being present in greater densities at one or more sites.

INTRODUCTION

Most of the information characterizing baseline conditions of the Colstrip study sites has been reported in the Third Interim Report (Lauenroth *et al.*, 1978). The purpose of this section is to report information that was not available at the time the earlier report was prepared. This section does not repeat information in detail, but does make reference to it. New information being reported is largely on abiotic and invertebrate characteristics.

SOIL AND TEMPORAL DYNAMICS OF SOIL WATER AND PRECIPITATION

Soils

Soils of the Colstrip sites have been described in a previous interim report (Lauenroth *et al.*, 1978). The soils of the areas are of three series--Kobar clay loam, Lonna loam, and Yamac silt loam and differ among the four study sites. Physical and chemical characteristics of the soils have been determined through analyses of samples taken from pits excavated on each study site (Appendix Tables 2.1-2.4).

Soil Water and Precipitation

Seasonal dynamics of soil water and growing season precipitation were monitored for the Colstrip sites in 1975 but not in 1974 and were reported in a previous interim report (Lauenroth *et al.*, 197). Soil water monitoring was discontinued in 1976 and precipitation for 1976 are reported in Appendix Table 2.5.

PRODUCERS AND PRIMARY PRODUCTION

Herbage and Litter Biomass Dynamics and Plant Phenology

Aboveground biomass dynamics for 1974 and 1975 were reported in the third interim report (Lauenroth *et al.*, 1978) and are not repeated in this report. Seasonal biomass dynamics were not monitored during 1976 and 1977. Sampling of herbage components for the Colstrip study sites was limited to one sample date in 1976 and in 1977. The single sample date was chosen to coincide with the peak of live standing crop by examination of the 1974 and 1975 data. Data from the single harvest date are discussed in connection with net primary production estimates for the sites (see section entitled Colstrip Sites Net Primary Production).

Crown and belowground biomass dynamics for the four Colstrip sites were determined in 1974 and 1975, and belowground biomass was determined for one date in 1977. The estimates of crown biomass are quite variable but do indicate an overall similarity in mean standing crop among the four study sites (Table 2.1). Significant temporal changes in crown biomass are not apparent.

TABLE 2.1. CROWN BIOMASS DYNAMICS, COLSTRIP STUDY SITES, 1974-1975* (G · M^{-2} ASH FREE)

Date	Hay Coulee		Kluver West		Kluver East		Kluver North	
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
11 May 1974	70.5	19.6	76.4	20.3	53.8	10.5	41.6	6.4
11 June 1974	72.6	20.5	56.0	11.1	58.0	18.3	30.9	6.7
29 June 1974	77.4	14.2	60.4	12.3	53.5	17.2	55.5	14.1
27 July 1974	29.7	3.8	55.0	14.4	39.2	7.6	35.4	3.9
16 August 1974	12.9	4.5	25.2	7.5	30.1	11.6	10.5	1.6
26 September 1974	68.4	14.6	77.3	14.8	87.1	10.3	43.6	6.7
20 April 1975	59.5	10.1	189.5	26.5	45.6	9.8	47.6	15.4
17 May 1975	88.2	16.1	59.0	14.6	67.3	16.3		
23 June 1975	41.6	6.2	47.6	10.1	50.5	11.6	29.1	4.1
15 July 1975	64.5	12.2	85.9	26.0	58.7	15.1	47.8	13.3
11 August 1975	49.2	20.1	99.9	44.1	70.8	24.9	43.2	14.5
17 September 1975	90.0	24.5	99.2	13.2	100.6	34.2		

---

*Crown data were not collected in 1976 and 1977.

Belowground biomass dynamics were based on measurements of plant parts (roots and rhizomes) in the surface 10 cm of the soil profile (Table 2.2). Previous analyses indicated that this layer includes about 50% of total belowground plant biomass (Lauenroth *et al.*, 1978). Belowground biomass estimates ranged from <400 to >600 g · m^{-2} over the four study sites, but consistent differences among study sites were not apparent. A seasonal trend of decreasing biomass from early spring and summer to late summer was found for nearly all sites in both years. The belowground biomass data utilized here include both live and dead material. Therefore, seasonal dynamics do not necessarily reflect live dynamics, but rather reflect the balance between additions through growth and losses through decomposition.

Litter dynamics were similar across the study sites (Table 2.3). Although within-season dynamics are inconsistent among years and treatments, all four sites have about the same amount of litter standing crop. There also appears to be a general increase in litter standing crop from 1974 to 1977 on all sites except Hay Coulee.

Colstrip Sites Net Primary Production

Estimates of net primary production for the Colstrip Site from 1974 through 1977 were obtained by summing the peak standing crops of current years production for each of the five functional groups (Table 2.4). In 1976 and 1977, the sites were clipped only once, on dates that we believed corresponded fairly well to the occurrence of peak aboveground standing crop. These data are difficult to analyze due to the occurrence of extreme increases in grasshopper populations on some of the sites in some of the years. The area most affected was the Kluver West site from 1975 through 1977, although Kluver North was also affected. The precipitation in this area varied significantly over the years (much lower in 1977 than in 1974, 1975, or 1976), further complicating the pattern of net primary production. The cool season grasses group was the major component of total net production in each year on all study sites. The grasshopper and precipitation fluctuations make it impossible to draw any conclusions about the short-term effects of power plant emissions on net primary production.

Chemical Composition of Major Plant Species

During the field sampling periods in 1974 and 1975 subsamples of all plant materials that were harvested in the production studies were prepared for chemical analysis. These samples were dried, ground, and stored in plastic vials in a constant temperature (≈23°C) room at the Natural Resource Ecology Laboratory.

Routine analyses for dry matter, ash, nitrogen, phosphorus, and total sulfur were made on the major species within 6 months of collection. The remainder of the plant materials in the tissue bank will be preserved for future reference. Although many of the chemical constituents will change with storage time, some will not. The bank should prove valuable in future studies designed to compare baseline chemical characteristics with chemical

TABLE 2.2. BELOWGROUND BIOMASS DYNAMICS* (0-10 CM, G · M^{-2} ASH FREE)

Date	Hay Coulee		Kluver West		Kluver North		Kluver East	
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
11 May 1974	576.6	42.0	555.6	41.5	472.7	57.2	425.4	33.6
11 June 1974	411.2	35.8	425.5	32.8	467.1	61.9	442.2	36.2
29 June 1974	468.3	21.9	485.6	27.8	492.2	43.7	493.7	22.4
27 July 1974	403.8	39.4	563.6	65.4	459.6	25.6	427.6	18.1
16 August 1974	395.5	34.7	412.0	37.8	484.7	63.8	433.2	21.0
26 September 1974	439.6	41.1	419.8	34.4	351.3	63.8	333.0	19.6
20 April 1975	428.4	54.1	611.8	46.3	642.5	53.5	439.7	35.6
17 May 1975	548.9	25.8	529.3	64.5	570.8	77.2		
23 June 1975	549.7	76.8	512.2	50.0	588.3	95.8	461.4	33.3
15 July 1975	470.1	35.6	452.8	43.7	447.9	39.3	424.7	23.7
11 August 1975	385.6	58.2	359.4	35.9	378.4	35.7	371.1	19.2
17 September 1975	318.0	23.7	491.5	25.3	505.9	90.5		
18 July 1977	419.3	38.4	459.8	39.2	491.5	29.6	473.1	31.3

---

*Belowground biomass data were not collected in 1976.

TABLE 2.3. LITTER STANDING CROP ($\bar{X}$ ± SE, ASH FREE G · M^{-2})

Date	Hay Coulee		Kluver West		Kluver North		Kluver East	
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
11 May 1974	161	17	177	13	234	21	158	16
11 June 1974	172	11	175	12	157	22	154	12
29 June 1974	201	13	183	6	169	15	171	19
26 July 1974	172	12	140	12	171	18	161	18
16 August 1974	128	12	139	19	198	10	153	18
26 September 1974	192	15	167	13	230	24	148	13
$\bar{X}$	171		163		193		157	
22 April 1975	162	10			193	22	144	17
18 May 1975	170	14	159	9	205	19		
20 June 1975	179	11	166	20	163	16	157	19
15 July 1975	154	15	160	10	218	32	152	14
11 August 1975	231	26	207	15	277	33	204	16
16 September 1975	218	18	191	14	189	16	206	39
$\bar{X}$	186		177		207		144	
29 June 1976	181	16	195	18	235	15	200	13
18 July 1977	227	14	313	14	313	14	310	18

TABLE 2.4. PEAK STANDING CROPS*

Functional groups	Hay Coulee	Kluver West	Kluver North	Kluver East
Cool season grasses				
1974	67.8 ± 5.8	103.2 ± 8.2	57.6 ± 5.1	60.8 ± 3.4
1975	94.5 ± 6.9	106.3 ± 10.7	73.6 ± 5.8	91.8 ± 5.7
1976	75.5 ± 4.1	55.6 ± 6.9	70.7 ± 6.0	95.1 ± 5.8
1977	36.2 ± 2.3	33.5 ± 2.3	22.5 ± 2.1	32.9 ± 3.7
Warm season grasses				
1974	16.4 ± 4.1	4.1 ± 1.6	14.1 ± 3.2	6.1 ± 2.6
1975	14.5 ± 3.4	9.6 ± 2.1	7.3 ± 3.5	8.5 ± 5.1
1976	14.9 ± 2.6	2.9 ± 1.2	6.6 ± 2.1	2.1 ± 1.0
1977	4.8 ± 1.5	1.7 ± 1.0	1.9 ± 0.8	1.3 ± 0.8
Cool season forbs				
1974	10.5 ± 2.3	9.3 ± 2.1	14.0 ± 1.2	9.0 ± 3.8
1975	15.3 ± 2.4	14.7 ± 4.3	25.8 ± 2.2	12.1 ± 2.9
1976	18.2 ± 2.6	10.2 ± 1.9	32.0 ± 2.4	14.0 ± 2.2
1977	6.4 ± 2.4	4.4 ± 1.4	11.1 ± 1.7	3.8 ± 1.8
Warm season forbs				
1974	0.0 ± 0.0	3.3 ± 1.4	0.0 ± 0.0	0.0 ± 0.0
1975	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1976	0.7 ± 0.7	2.1 ± 2.1	0.0 ± 0.0	0.6 ± 0.6
1977	1.4 ± 1.4	0.4 ± 0.4	0.0 ± 0.0	0.3 ± 0.3
Half shrubs				
1974	13.1 ± 4.4	0.0 ± 0.0	37.0 ± 5.2	29.6 ± 6.3
1975	14.6 ± 5.4	0.0 ± 0.0	36.3 ± 7.9	52.4 ± 10.7
1976	7.8 ± 5.1	0.0 ± 0.0	9.3 ± 1.7	8.5 ± 2.3
1977	1.5 ± 0.6	0.0 ± 0.0	10.3 ± 2.7	1.6 ± 0.7
Aboveground net production				
1974	107.8	119.9	122.7	105.5
1975	138.9	130.6	143.0	164.8
1976	117.1	70.8	118.6	120.3
1977	50.3	40.0	45.8	39.9

---

*Current and aboveground net primary production ($\bar{X}$ ± SE). Peaks for 1976 and 1977 estimated from one harvest date only, 28 June 1976 to 16 July 1977.

characteristics of plants from the same study sites 5 or 10 years after continuous operation of the Colstrip power plant complex.

Differences in chemical constituents among the four study sites for live *Agropyron smithii*, the dominant grass, are minimal (Table 2.5). Ash content ranges from 5.6% to 9.4% but is usually about 7%, regardless of site of time of season. By contrast, nitrogen content ranges from above 2% in May to less than 1% in August. Although the sulfur content of live *Agropyron smithii* also appears to decrease with advance of the season, it does not decrease as rapidly as does foliar nitrogen. Little can be said of the intraseasonal changes in phosphorus concentrations from our sparse 1974 data and we did not determine phosphorus levels in 1975.

INVERTEBRATE POPULATIONS*

Introduction

This section will present the results of arthropod censusing during the 1974 and 1975 field seasons. The field sampling was done on the four established study plots near Colstrip, i.e., Hay Coulee, Kluver West, Kluver North, and Kluver East. A general description of the region has been given by Lauenroth *et al.* (1975) and Taylor *et al.* (1976) has given detailed descriptions of the four study plots. For convenience, Taylor's descriptions are summarized in Table 2.6.

There have been very few studies of the total arthropod community of northern mixed grass prairie systems (McDaniel, 1971; Reigert and Varley, 1972; Willard, 1974; Reigert *et al.*, 1974). This study, in addition to the primary purpose of providing baseline data for the coal-fired power plant emissions study, is an attempt to provide a detailed description of the arthropod community of a representative site within the northern mixed grass prairie.

Methods and Materials

The field and laboratory techniques were discussed in brief previously in the Third Interim Report, but will be given here in greater detail for reference purposes. The techniques are the same as those followed at Pawnee Site, the primary field research site of the US/IBP Grassland Biome project. Specific details of all equipment and procedures are given by Leetham (1975).

The arthropod community was sampled in three phases by three different techniques, each of which was directed at a major component of the community. The three phases were aboveground arthropods, soil macroarthropods, and soil

---

*Excerpted from J. W. Leetham, T. J. McNary, R. Kuhmar, J. Lloyd, R. Lavigne, J. L. Dodd and W. K. Lauenroth. 1978. Arthropod consumer dynamics in some mixed-prairie grasslands in the northern Great Plains. (In preparation)

TABLE 2.5. CHEMICAL CONSTITUENTS OF LIVE *AGROPYRON SMITHII* PLANT SAMPLES FOR COLSTRIP STUDY SITES

		Ash (%)		Nitrogen (%)		Phosphorus (%)		Sulfur (%)	
Site	Date	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
Hay Coulee	29 June 1974	6.9	6.4	1.3	1.2	.19	.17	.09	.10
	16 August 1974	6.4	4.5	0.9	0.8	.13	.10	.07	.07
	1 May 1975	9.4	8.1	2.5	2.6			.11	.10
	1 June 1975	7.2	7.3	1.5	1.6			.10	.11
	15 July 1975	7.4	7.6	1.1	1.1			.07	.06
	11 August 1975	6.9	6.8	0.8	0.8			.06	.06
	28 June 1976	7.1	6.6	1.1	1.2			.08	.08
Kluver West	30 June 1974	6.4	5.6	1.1	1.3	.18	.14	.07	.10
	22 August 1974	6.6		0.9		.10		.07	
	1 May 1975	7.4	8.2	2.1	2.3			.07	.09
	1 June 1975	7.1	6.3	1.4	1.5			.09	.09
	16 July 1975	7.0	7.7	1.1	1.1			.05	.08
	12 August 1975	6.5	6.1	0.8	0.9			.06	.06
	29 June 1976	6.7	6.1	1.1	1.1			.07	.08
Kluver North	1 July 1974	5.9	5.8	1.2	1.3	.12	.15	.09	.09
	12 August 1974	6.3	6.3	0.8	1.0	.10	.09	.10	.10
	1 May 1975	7.4	7.6	2.4	2.4			.10	.10
	1 June 1975	7.1	6.6	1.5	1.5			.09	.10
	1 July 1975	8.4	6.4	1.2	1.2			.06	.10
	12 August 1975	6.0	7.5	0.8	1.0			.07	.08
	29 June 1976	6.4	6.4	1.2	1.3			.08	.08

TABLE 2.5. CONTINUED.

Site	Date	Ash (%)		Nitrogen (%)		Phosphorus (%)		Sulfur (%)	
		Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
Kluver East	2 July 1974	8.5	7.6	1.2	1.3	.17	.16	.11	.11
	13 August 1974	8.3		0.9		.10		.08	
	1 June 1975	7.0	6.6	1.5	1.4			.07	.08
	17 July 1975	7.6	7.6	1.2	1.1			.05	.08
	30 June 1976	7.7	7.1	1.2	1.3			.07	.07

TABLE 2.6. GENERAL CHARACTERISTICS OF THE FOUR FIELD SITES NEAR COLSTRIP, MONTANA*

Field plot	Distance (km) and direction from Colstrip	Elevation (m)	Slope and exposure	Principal plants	Soil type
Hay Coulee	11.6 SE	927	4% NNE	*Agropyron smithii* *Artemisia tridentata* *Koeleria cristata* *Bromus japonicus*	Colluvial clay loams
Kluver West	11.6 ESE	917	6% N	*Stipa comata* *Agropyron smithii* *Bromus japonicus* *Bromus tectorum*	Colluvial sandy loams
Kluver North	14.7 E	902	5% NE	*Stipa comata* *Artemisia frigida* *Agropyron smithii* *Bromus japonicus*	Colluvial sandy loam
Kluver East	18.3 ESE	904	3.5% NE	*Agropyron smithii* *Agropyron cristata* *Artemisia frigida* *Bromus japonicus*	Residual and colluvial clay loams

---

*Derived from Taylor *et al.*, 1975.

microarthropods. The aboveground arthropods included those individuals occurring in or above the soil surface litter. The soil macroarthropods included those individuals occurring below the surface litter and incapable of passing through a 1-mm opening sieve while the soil microarthropods were those individuals occurring in or below the surface litter material and small enough to pass through the 1-mm sieve. Because of difficulty in applying the latter, physical separation of the soil macro- and microarthropods, the separation was ultimately based on taxonomic criteria. Thus the soil microarthropods included the soil acarines, primitive or apterygote insects, Pauropoda, Symphyla, Tardigrada, and one "higher" group of insects, the Homoptera-Pseudococcidae. The soil macroarthropods included the remainder of the soil-dwelling arthropods. The surface litter material was included in the microarthropod samples because we felt the very small individuals occurring there were poorly represented in the litter material in the aboveground samples while the opposite argument was applied to the macroarthropods.

The aboveground arthropods were sampled by dropping a cage of known size over predetermined sample locations without disturbing the arthropods. The trap, covering a 0.5-m^2 circular area, was dropped from a 5.5 m (18 ft) cart-mounted boom. After trap emplacement, the arthropods were removed by vacuum in a two-stage process designed to increase the efficiency of sampling. In the first stage the trap and vegetation were vacuumed lightly to remove the active arthropods without including a lot of litter. The second stage was the complete removal of all vegetation and litter by clipping and vacuuming. The first-stage material was frozen and later handsorted while the second stage material was subjected to Berlese-type extraction for 48 h for removal of the arthropods which were killed and preserved in 70% ethanol.

The soil macroarthropods were sampled by taking 12.5 cm (5 ft) diam. by 15 cm deep soil cores and wet sieving them through a series of three sieves with mesh openings of 4, 2, and 1 mm. The retained material was then suspended in a saturated solution of magnesium sulfate ($MgSO_4$) to separate organic and inorganic material. The final material was handsorted for arthropods. The soil cores were taken within the area where the aboveground sample was taken.

The soil microarthropods were sampled by taking soil cores 4.8 cm diam. by 10 cm deep in such a way as to minimize soil compaction and retain the core in two 5-cm long aluminum sleeves. The core was taken outside the area used for the aboveground sample where the litter had not been removed. The soil core was divided into the two 5-cm sections and each was later subjected to Macfadyen-type high-temperature gradient Tullgren extraction as developed by Merchant and Crossley (1970). The arthropods were killed and preserved in 70% ethanol. In addition to the 10-cm deep cores, one sampling was made to 60-cm depth on the Kluver East plot in April 1975. The purpose of this sampling was to determine the vertical distribution of the soil microarthropods. The 60-cm cores were divided into and extracted by 5-cm increments. The extraction period for each core segment was 7 days which allowed for complete desiccation of the sample.

All aboveground arthropod and soil macroarthropod samples were sorted and identified at the University of Wyoming, Laramie, while the soil microarthropod samples were processed at Colorado State University, Fort Collins. For all sample types, the arthropods were identified to at least family and, where possible, to genus and species, and each type was assigned to a trophic or functional grouping. Representative specimens or groups of specimens of each taxonomic group were dried at approximately 65°C for a minimum of 24 h then weighed for dry weight biomass. Total counts and biomass determinations were projected to a m^2 basis.

The trophic categories used were: root tissue feeder, root sap feeder, fungivore, aboveground plant tissue feeder, aboveground plant sap feeder, pollen and nector feeder, seed feeder, predator, parasitoid, omnivore, scavenger, non-feeding, and unknown. A detailed discussion of these groups is given by McDaniel (1971). Assignment of the various arthropods to these trophic groups was based on literature reference, gut content analysis, and/or recommendations by authorities to whom representatives were sent for identification and/or verification. In cases where immature stages differed from adults in feeding habits, the general trophic assignment was based on which stage had the greatest effects on the total system. An example is the parasitic Hymenoptera where the parasitoid larvae are probably exerting a much greater effect than non-feeding or nectar feeding adults; therefore, all specimens were given the "parasitoid" assignment.

The frequency and magnitude of sampling was essentially the same in each of the two years of field work. The number of sampling dates for each group in each year is presented in Table 2.7. No samples were taken in the dormant or winter season. Each field plot was divided into two replicates and each was gridded into 1-m^2 sampling subunits. At each field date, five sampling units were chosen in each replicate (10 per field plot) by use of a random numbers table. Within each sampling unit, one sample for each major arthropod category was taken, i.e., one drop trap sample and one macro- and one microarthropod soil core. Beginning with the third field date in 1975 (13 June 1975) two macroarthropod cores were taken at each sample location. The resulting data were summarized by replicate and treatment (field plot).

Results and Discussion

The present count of identified arthropod types collected during the two seasons of field sampling includes 100 families of insects representing 17 orders, 54 families of the four major suborders of mites (Acarina), four families of spiders (Araneida), one family of Chilopoda, one species of terrestrial Copopod (unidentified to species), Pauropoda, Symphyla, and Tardigrada. Not all material collected has been identified to genus or species or, in some cases to family, and some material is undescribed (particularly in the Acarina). A general overview of the taxonomic structure of the arthropod community is presented in Figure 2.1. The percentages used were derived by combining the results of all three types of sampling averaging over time and across field plots. Biomass was used because we felt it gave a better representation of the community structure than numbers since the overwhelming numbers of Acarina would have made the remaining taxa appear insignificant. Also presented is the relative contribution to the total by each portion of the community (i.e., aboveground and soil micro- and

TABLE 2.7. FIELD SAMPLING DATES FOR ARTHROPODS FOR 1974 AND 1975

Aboveground arthropods		Soil macroarthropods		Soil microarthropods	
1974	1975	1974	1975	1974	1975
11 May*	21 April*	14 May	19 April	14 May*	19 April
25 June*	23 May*	23 June	19 May	16 June*	18 May*
5 July*	20 June*	19 July*	13 June*	--	20 June*
1 Aug.*	14 July*	12 Aug.*	23 July*	9 Aug.*	17 July*
18 Aug.*	8 Aug.*	19 Aug.*	6 Aug.*	20 Aug.*	12 Aug.*
12 Sept.*	11 Sept.*	11 Sept.*	17 Sept.*	12 Sept.*	16 Sept.*

*Sampling dates from which data were used for statistical analysis.

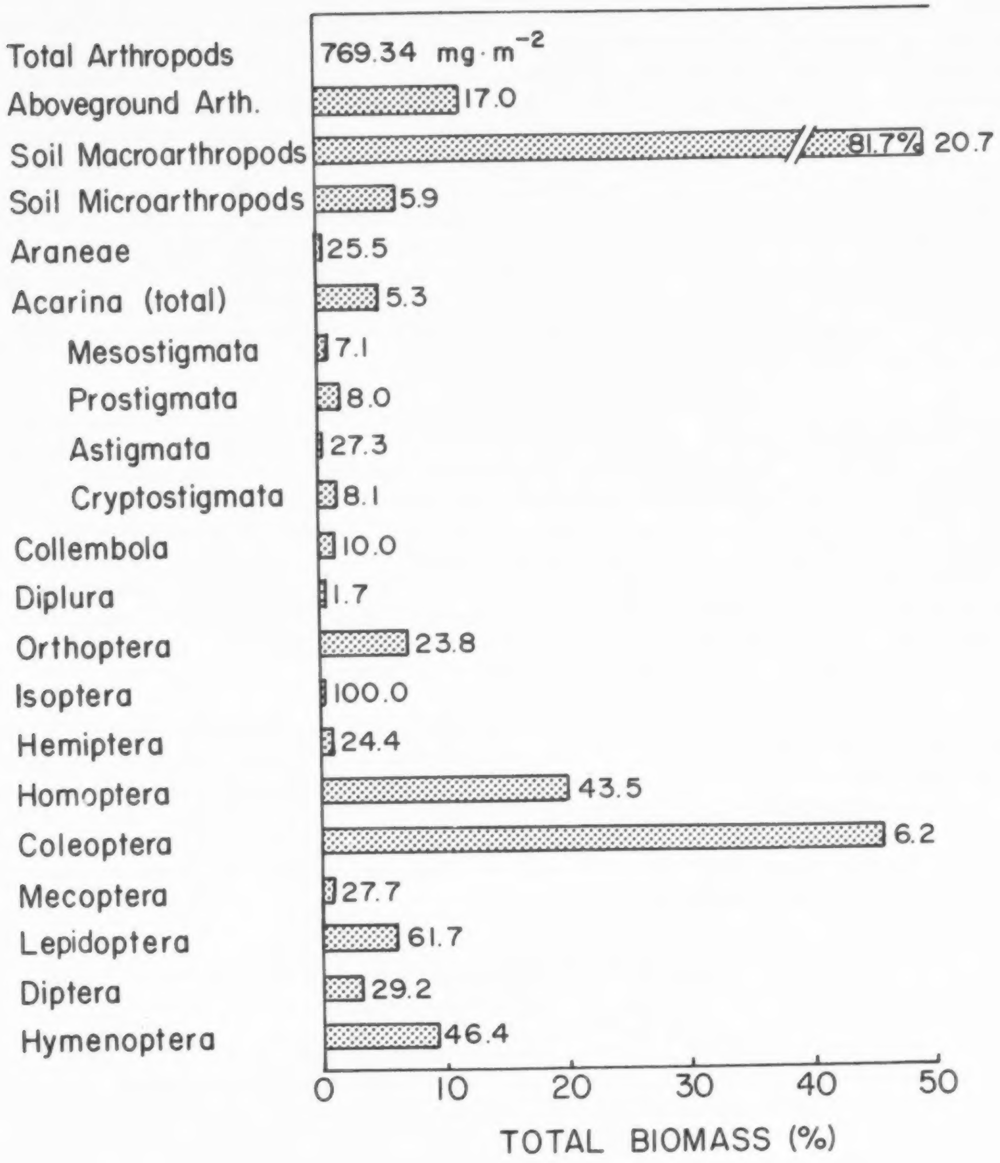


Figure 2.1. General taxonomic structure of the arthropod community of a southeastern Montana mixed-grass prairie, based on averaging time-weighted means from each of the four field plots from the 1974 and 1975 seasons for biomass. Numbers indicate standard error as a percent of the total.

macroarthropods). The figures listed in the right column are the standard error given as a percentage of the mean of the four plots. These data are given to provide an indication of the variability between field plots. Only the major taxa are given in Figure 2.1. Those collected but not shown include Chilopoda, Pauropoda, Symphyla, Tardigrada, Plecoptera, Protera, Psocoptera, Thysanoptera, Tricoptera, Neuroptera, and Odonata. The Coleoptera is the dominant group and one of the most stable across sites. The Acarina are also quite stable across the four field sites, while most of the insect orders show high variability. A vast majority of the total insect biomass occurs in the soil and surface litter (approximately 88%). A more detailed breakdown of the insect community on each of the four field sites is given in Table 2.8. The very high biomass on the Hay Coulee site was caused by collection of Cicada immatures (Homoptera-Cicadidae) just before they emerged. Normally, those immatures are known to occur at depths of 40-60 cm for most of their developmental stages.

Figure 2.2 is a general trophic breakdown of the insect community at the four field sites. The figures are presented for the three major groupings or sampling schemes. All herbivore types were combined for simplification. Herbivory is the predominant feeding type in both aboveground and soil macroarthropod groups but ranks only third in the soil microarthropods. Predation, however, is very important in both soil groups but is not nearly as important above ground. Fungal feeding is the predominant feeding type in the soil microarthropods. The soil macroarthropod data are the most variant across the four sites while the soil microarthropod and aboveground arthropod data are similar in their variability across sites.

In order to determine possible significant differences between the four sites in the various components of the arthropod community as well as significant year differences or seasonal fluctuations a repeated measures analysis of variance was used. The analysis was applied to each of the three arthropod sampling components and the model used was the same except for the sample dates for each since some variability in the number and spacing of the dates occurred. The model used was:

$$Y_{ijkp} = \mu + t_i + \pi_{p(i)} + z_j + d(z)_{k(j)} + tz_{ij} + td(z)_{ik(j)} + \varepsilon_{ijkp}$$

where t = treatment and $i = 1, \ldots, 4$ (1 = Hay Coulee, 2 = Kluver West, 3 = Kluver North, and 4 = Kluver East); π = replicate within treatment and $p = 1, 2$ for each; z = year and $j = 1, 2$ (1 = 1974, 2 = 1975); $d(z)$ = date within year and $k = 1, \ldots, n$ for $j = 1$; $k = 1, \ldots,$ for $j = 2$; and n = the total number of dates used for a given response; and ε = error = $\pi z + \pi d(z)$. The sampling dates used in each of the three tests are indicated in Table 2.7. Additionally, certain soil macroarthropod groups were tested using only the last four dates in 1975. The model for this test was:

$$Y_{ikp} = \mu + t_i + \pi_{p(i)} + d_k + td_{ik} + \varepsilon_{ikp}$$

TABLE 2.8. GENERAL STRUCTURE OF THE ARTHROPOD COMMUNITY OF THE FOUR FIELD SITES*

Arthropods	Hay Coulee	Kluver West	Kluver North	Kluver East
		Numbers · m^{-2}		
Aboveground arthropods	79 (0.1)[†]	62 (0.1)	101 (0.1)	70 (0.1)
Soil macroarthropods	139 (0.2)	184 (0.2)	87 (0.1)	93 (0.1)
Soil microarthropods	85,905 (99.7)	118,039 (99.7)	117,787 (99.8)	90,954 (99.8)
Total	86,123	118,284	117,975	91,117
		Biomass (mg · m^{-2})		
Aboveground arthropods	70.6 (6.3)	130.3 (17.2)	101.1 (16.7)	62.7 (10.8)
Soil macroarthropods	1,012.7 (89.7)	571.2 (75.4)	456.0 (75.2)	476.4 (81.8)
Soil microarthropods	45.5 (4.0)	55.7 (7.4)	49.6 (8.1)	43.3 (7.4)
Total	1,128.8	757.2	606.7	582.4

*Based on means of 1974 and 1975 time weighted means except for soil macroarthropods for which only 1975 data are used.

[†] Percent of total.

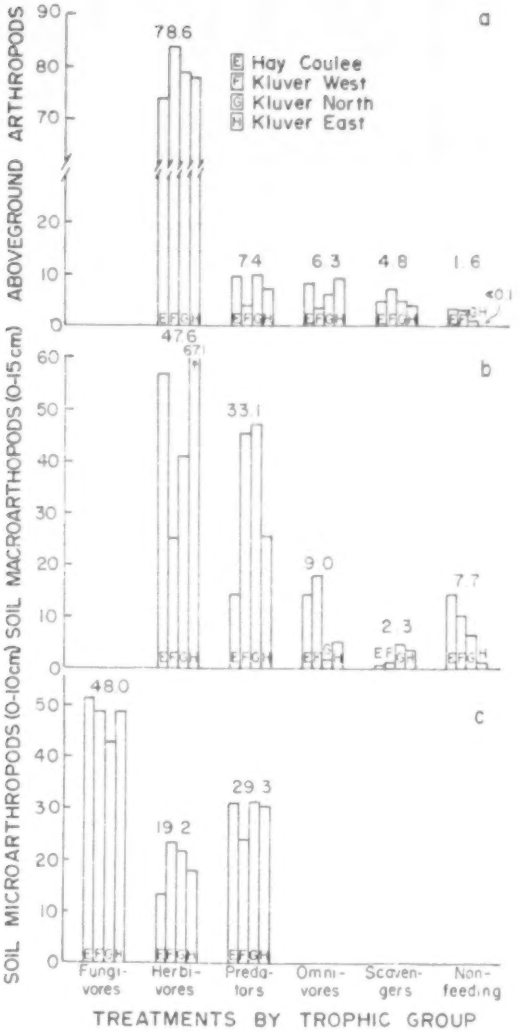


Figure 2.2. Trophic structure of the arthropod community on the four field sites. Percentages are based on means of 1974 and 1975 time-weighted means except the soil macroarthropods for which only 1975 data are used. Numbers above bars indicate mean of all four treatments.

where the terms and subscripts are the same as before, except d = date for 1975 only and $k = 1, \ldots, 4$ (for last four sampling dates of 1975) and $\varepsilon = \pi d$.

For the other groups of soil macroarthropods tested, only the last four dates in both 1974 and 1975 were used. The deletion of certain dates was because of problems in the field and laboratory techniques that were corrected at the third sampling in 1975, thus making the last four samplings in 1975 the most reliable data. For the soil microarthropods, only five of the six 1975 samplings were used since they corresponded with the five samplings of 1974, thus giving the test for year effect more validity. Only the major taxonomic and trophic groups were tested and each set of data (aboveground arthropods and soil micro- and macroarthropods) was treated separately. The analyses were designed to test for year, treatment, and date main effects and interactions. The results of the statistical tests (including means) are presented in Tables 2.9-2.17. Only the results for biomass are presented since, as mentioned previously, we feel they represent the faunal structure better than those for numbers. Where the results for numbers deviate from that for biomass, they will be discussed in text.

Of the 14 taxonomic and trophic groups (including total) of aboveground arthropods tested, only the Coleoptera failed to show a significant change in numbers and/or biomass from 1974 to 1975 ($P \leq 0.05$). Of those that changed, only the Araneida numbers and scavenger biomass showed a decrease in population size from 1974 to 1975. It is suspected by the authors, that increased efficiency of sampling in 1975 may have had significant effects on the observed results of increased populations in 1975 since many logistical problems of the initial 1974 season had been largely overcome by 1975.

Significant differences between the aboveground arthropod populations of the four plots were few. For $P \leq 0.05$, only the Hemiptera and the trophic groups of scavengers, plant sap feeders, and predators showed different populations. Three groups, i.e., total, Orthoptera, and omnivores, had significant differences at $0.05 < P < 0.10$; however for numbers, the Orthoptera was highly significant ($P < 0.01$). There were some significant interactions noted and these data are presented in Figures 2.3 and 2.4. The high orthopteran populations at the Kluver West plot are due to high grasshopper (mostly *Melanoplus* sp.) populations in 1975. The rancher on whose land the plot is located confirmed that the area around the plot is a notorious grasshopper outbreak area. It can be seen in Figures 2.3a,b that there were substantially higher grasshopper populations in 1975 than in 1974. Very high populations have been noted in this same plot in 1976 and 1977, although no sampling was done. The high Hemiptera and resulting plant sap-feeding populations on Kluver North were due to the lygaeid *Nysius* sp. collected in very high numbers primarily in May and June of both years.

As was mentioned earlier, certain difficulties with both the field and laboratory techniques used for soil macroarthropods caused the data from 1974 and early 1975 to be less reliable than desired and hence the significant population increases in 1975 as noted in Table 2.10 are most probably artifical. The data from the last four sampling dates in 1975 are considered reliable. Because of these difficulties we cannot make any conclusions as to

TABLE 2.9. ANOVA RESULTS FOR ABOVEGROUND ARTHROPOD-BIOMASS (MG · M^{-2})-YEAR AND TREATMENT EFFECTS

Aboveground arthropods	Year		ANOVA	Field plot				ANOVA results		
	1974	1975	P(Y)	Hay Coulee	Kluver West	Kluver North	Kluver East	P(T)	Q value	P(T×Y)
Araneida	2.45	1.60	.3472	2.60	1.08	3.33	1.10	.0168	1.829	
Orthoptera	11.92	79.76	.0000	31.72	76.80	43.61	31.22	.0738		.0482
Hemiptera	3.97	5.54	.0419	3.02	5.27	8.30	2.42	.0284	5.04	
Homoptera	4.16	6.70	.0061	6.35	5.43	6.08	3.86	.3894		
Coleoptera	18.47	15.89	.2327	13.31	20.32	23.24	12.39	.2172		
Hymenoptera	2.76	8.33	.0000	6.00	4.88	6.40	4.89	.4755		
Diptera	0.93	1.68	.0087	1.31	1.32	1.44	1.14	.9675		
Plant tissue feeders	30.05	94.24	.0000	43.95	92.92	67.55	44.17	.1412		
Plant sap feeders	7.75	9.57	.0906	7.25	9.52	12.69	5.18	.0216	5.63	.0188
Plant pollen feeders	0.36	2.58	.0069	9.93	2.98	0.83	0.13	.1572		
Predator	5.71	5.35	.7720	5.84	4.43	7.91	3.94	.0483	3.95	
Omnivore	1.84	7.24	.0000	5.10	3.38	5.38	4.67	.0732		
Scavenger	5.85	2.82	.0007	2.76	8.17	4.18	2.23	.0033	6.73	.0330
Total arthropods*	53.13	126.13	.0000	70.53	125.06	100.82	62.10	.0943		.0078

*Total aboveground arthropods, but does not necessarily equal the sum of those listed.

TABLE 2.10. ANOVA RESULTS TO SOIL MACROARTHROPODS-BIOMASS (MG · M^{-2})-YEAR AND TREATMENT EFFECTS

Aboveground arthropods	Year		ANOVA	Field plot				ANOVA results		
	1974	1975	P(Y)	Hay Coulee	Kluver West	Kluver North	Kluver East	P(T)	Q value	P(T×Y)
Orthoptera	12.30	4.56	.1169	1.57	4.30	18.00	9.87	.1671		.0253
Coleoptera	86.74	324.68	.0000	238.44	225.04	197.65	161.72	.6799		
Hymenoptera	9.13	63.77	.0478	67.57	62.34	11.35	7.55	.5124		
Plant tissue feeders	42.94	123.96	.0130	132.28	73.05	39.99	88.48	.7125		
Homoptera		132.53		275.67	21.22	69.32	163.93	.3450		
Root tissue feeders		183.10		247.05	120.14	180.58	184.63	.9367		
Root sap feeders		295.21		551.34	42.44	199.10	387.97	.4752		
Predators		343.63		264.16	508.53	407.34	194.49	.0279	265.79	
Total arthropods*	125.73	614.01	.0000	567.15	352.14	286.70	273.50	.0429	290.53	

*Total soil macroarthropods, but does not necessarily equal the sum of those listed.

TABLE 2.11. ANOVA RESULTS FOR SOIL MICROARTHROPODS-BIOMASS (MG · M^{-2})-YEAR AND TREATMENT EFFECTS

Aboveground arthropods	Year		ANOVA	Field plot				ANOVA results		
	1974	1975	P(Y)	Hay Coulee	Kluver West	Kluver North	Kluver East	P(T)	Q value	P(T×Y)
Acariformes	28.74	22.51	.0014	23.14	28.29	26.57	24.38	.2306		
Astigmata	1.65	0.65	.0026	0.64	1.10	1.86	1.00	.3031		
Cryptostigmata	13.76	7.70	.0000	10.53	12.69	9.22	10.48	.1598		
Prostigmata	13.32	14.15	.3512	11.97	14.59	15.58	12.89	.3205		
Parasitiformes	5.43	4.56	.1678	5.39	4.33	5.78	4.54	.2444		
Mesostigmata										
Fungivores	21.52	19.61	.3818	20.38	24.26	17.70	19.92	.3024		
Herbivores	8.16	11.30	.0199	6.27	13.47	10.56	8.62	.0796		.0570
Predators	14.84	10.44	.0000	13.07	12.07	13.08	12.34	.7824		
Total arthropods*	212.4	219.4	.9134	131.39	280.37	325.51	126.41	.4311		

*Total soil microarthropods, but does not necessarily equal the sum of those listed.

TABLE 2.12. ANOVA RESULTS FOR ABOVEGROUND ARTHROPODS-BIOMASS (MG · M^{-2})-DATE EFFECT FOR 1974

Aboveground arthropods	Sampling dates						ANOVA results		
	11 May	25 June	5 July	1 Aug	18 Aug	12 Sept	P(D)	Q value	P(D×T)
Araneida	1.92	1.83	4.88	1.78	2.23	2.10			
Orthoptera	5.65	24.18	12.04	14.89	12.84	1.91	.9421		
Hemiptera	7.21	10.68	2.58	0.37	0.52	2.44	.0000	5.490	.0000
Homoptera	2.66	8.70	5.55	2.59	3.51	1.93	.0287	6.466	
Coleoptera	16.96	32.85	26.16	5.57	7.94	22.98	.0001	17.214	.0147
Hymenoptera	4.06	6.79	3.70	0.22	0.46	1.30	.0024	5.140	
Diptera	0.31	2.05	2.11	0.27	0.58	0.26	.0079	1.988	
Plant tissue feeders	55.01	38.95	32.23	18.13	18.22	17.79	.5194		
Plant sap feeders	9.48	17.87	7.68	3.16	4.06	4.26	.0000	7.669	.0013
Plant pollen feeders	0.00	1.40	0.70	0.02	0.04	0.02	.9687		
Predators	4.53	8.92	9.65	2.43	3.03	5.69	.1049		
Omnivores	4.05	3.89	1.70	0.10	0.34	0.98	.0370	4.54	
Scavengers	6.23	13.90	4.94	1.95	3.63	4.47	.0000	6.037	.0000
Total arthropods*	81.32	89.21	58.34	26.04	29.56	33.81	.0257	68.79	

*Total aboveground arthropods, but does not necessarily equal the sum of those listed.

TABLE 2.13. ANOVA RESULTS FOR ABOVEGROUND ARTHROPODS-BIOMASS (MG · M^{-2})-DATE EFFECT FOR 1975

Aboveground arthropods	Sampling dates						ANOVA results		
	21 Apr	23 May	20 June	14 July	8 Aug	11 Sept	P(D)	Q value	P(D×T)
Araneida	0.24	0.28	5.59	2.49	0.16	0.86			
Orthoptera	1.78	14.58	35.25	147.43	192.95	86.60	.0000	66.522	.0037
Hemiptera	3.40	4.18	12.97	8.07	2.74	1.89	.0000	5.490	.0004
Homoptera	2.38	3.03	10.82	12.76	7.51	3.73	.0000	6.466	.0140
Coleoptera	29.93	29.64	19.76	8.56	3.15	4.31	.0000	17.214	.0517
Hymenoptera	3.05	13.24	19.45	11.56	2.04	0.67	.0000	5.140	.0005
Diptera	1.08	2.73	2.42	3.12	0.72	0.00	.0001	1.988	.0005
Plant tissue feeders	29.87	38.28	49.78	159.84	197.24	90.46	.0000	68.923	.0104
Plant sap feeders	3.55	4.51	22.02	14.30	9.49	3.53	.0000	7.669	.1090
Plant pollen feeders	1.96	0.25	4.23	3.52	1.96	3.54	.3410		
Predators	4.40	6.46	10.84	7.35	1.18	1.75	.0242	9.080	
Omnivores	2.80	12.26	19.36	8.78	0.64	0.67	.0000	4.54	.0001
Scavengers	3.48	6.01	5.13	0.22	0.35	1.74	.0228	6.037	.0329
Total arthropods*	47.91	69.43	127.36	196.99	213.15	101.92	.0000	68.79	.0093

---

*Total aboveground arthropods, but does not necessarily equal the sum of those listed.

TABLE 2.14. ANOVA RESULTS FOR SOIL MACROARTHROPODS-BIOMASS (MG · M^{-2})-DATE EFFECTS FOR 1974

Soil macroarthropods	Sampling dates				ANOVA results		
	19 July	12 Aug	19 Aug	11 Sept	P(D)	Q value	P(D×T)
Coleoptera	16.44	81.26	68.43	180.83	.2359		
Hymenoptera	11.80	12.68	4.98	7.07	.9486		
Orthoptera	0.00	10.16	0.00	39.06	.0008	26.11	.0141
Plant tissue feeding	0.00	13.34	50.16	108.27	.3074		
Total arthropods*	28.33	163.05	73.41	238.15	.4430		

---

*Total soil macroarthropods, but does not necessarily equal the sum of those listed.

TABLE 2.15. ANOVA RESULTS FOR SOIL MACROARTHROPODS-BIOMASS (MG · M^{-2})-DATE EFFECTS FOR 1975

Soil macroarthropods	Sampling dates				ANOVA results		
	20 June	30 July	8 Aug	11 Sept	P(D)	Q value	P(D×T)
Coleoptera	249.30	224.86	509.62	314.96	.0051	216.42	
Hymenoptera	74.21	96.85	62.48	21.54	.5513		
Orthoptera	13.86	2.52	0.63	1.26	.4773		
Plant tissue feeding	139.61	86.10	101.78	168.35	.5371		
Homoptera	120.95	362.86	45.97	0.37	.0174	302.06	.0181
Predator	301.11	243.90	648.17	181.34	.0159	385.63	
Root tissue feeding	214.32	128.84	344.71	44.53	.2495		
Root sap feeding	362.84	725.69	91.95	0.37	.0402	702.67	.0363
Total arthropods*	553.59	771.32	702.94	427.19	.0795		.0036

*Total soil macroarthropods, but does not necessarily equal the sum of those listed.

TABLE 2.16. ANOVA RESULTS FOR SOIL MICROARTHROPODS-BIOMASS (MG · M^{-2})-DATE EFFECTS FOR 1974

Soil microarthropods	Sampling dates					ANOVA results		
	14 May	16 June	9 July	20 Aug	12 Sept	P(D)	Q value	P(D×T)
Acariformes	39.47	33.54	25.72	17.86	27.08	.0001	11.587	
Astigmata	1.30	4.10	0.81	0.91	1.14	.0001	1.989	
Cryptostigmata	21.95	13.26	12.18	8.43	12.97	.0001	7.165	
Prostigmata	16.22	16.19	12.72	8.52	12.96	.0012	5.621	
Parasitiformes								
Mesostigmata	5.30	6.36	4.40	4.62	6.46	.4317		.0233
Fungivores	42.69	22.32	14.33	10.48	17.80	.0000	13.902	
Herbivores	9.00	7.90	7.67	5.21	11.02	.3808		
Predators	18.43	14.57	13.26	14.38	13.60	.0963		.0001
Total arthropods*	133,150	125,210	75,571	55,869	80,668	.0000	39,286	

*Total soil microarthropods, but does not necessarily equal sum of those listed.

TABLE 2.17. ANOVA RESULTS FOR SOIL MICROARTHROPODS-BIOMASS (MG · M^{-2})-DATE EFFECTS FOR 1975

Soil microarthropods	Sampling dates					ANOVA results		
	18 May	24 June	17 July	12 Aug	16 Sept	P(D)	Q value	P(D×T)
Acariformes	48.41	25.08	14.92	12.35	11.78	.0000	11.587	
Astigmata	1.78	0.64	0.23	0.14	0.48	.1458		
Cryptostigmata	14.72	8.71	5.54	3.71	5.83	.0008	7.165	
Prostigmata	31.90	15.73	9.15	8.49	5.46	.0000	5.621	.0048
Parasitiformes								
Mesostigmata	7.35	9.31	3.63	1.10	1.43	.0000	3.936	
Fungivores	62.62	15.75	7.64	5.18	6.85	.0000	13.902	
Herbivores	17.36	11.69	4.94	9.16	13.34	.0021	8.291	
Predators	14.98	20.78	9.34	4.34	2.74	.0000	5.78	
Total arthropods*	250,655	114,584	51,766	55,414	37,301	.0000	39.286	.0914

*Total soil microarthropods, but does not necessarily equal sum of those listed.

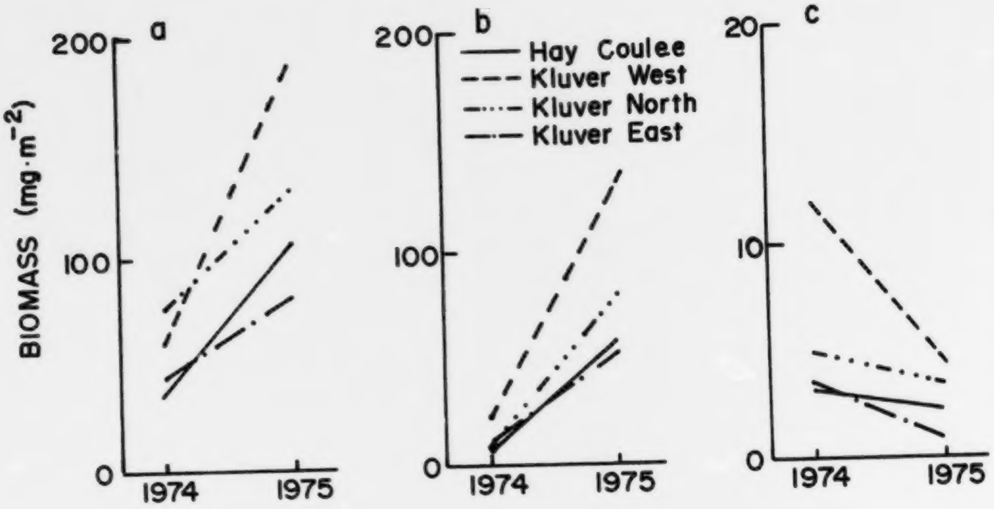


Figure 2.3. Treatment by year means (biomass) for three aboveground arthropod groups: a = total aboveground arthropods, b = Orthoptera, and c = scavengers.

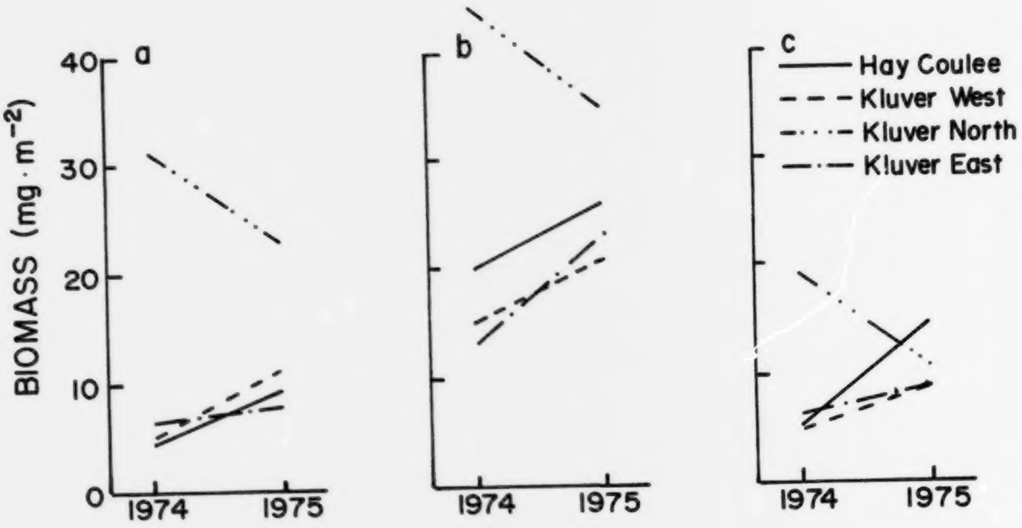


Figure 2.4. Treatment by year means (numbers) for three aboveground arthropod groups: a = Hemiptera, b = plant sap feeders, and c = omnivores.

population fluctuations of soil macroarthropods between the two years. Very little significant differences were found between the four field plots. Only the total (P = 0.0429) and the trophic group predator (P = 0.0279) showed significantly higher populations at one or more plots. Only the Orthoptera (primary grasshopper eggs) numbers and biomass (P = 0.0353 and 0.0253, respectively) showed a significant treatment × year interaction.

Seven of the eight microarthropod taxa and trophic groups tested showed significantly different ($P \leq 0.05$) populations from 1974 to 1975, most showing declines in 1975 (Table 2.10). Two exceptions were the prostigmatid numbers (P = 0.0303) and total herbivore number (P = 0.0039) and biomass (P = 0.0199) which showed increases in 1975. Total microarthropod numbers and biomass showed increases in 1975; however, neither were significant (P = 0.2066 and 0.9134, respectively). Except for possibly predators (P = 0.057) none of the groups tested showed significant year × treatment interactions, indicating the year differences are probably valid for all four field plots.

No significant differences between plots ($P \leq 0.05$) were found for any of the taxonomic and trophic groups of soil microarthropods tested, although variability between the plots is evident in the data (Table 2.10). At P = 0.0776 and P = 0.0796, the herbivore group showed significant treatment effect for numbers and biomass, respectively. The most apparent trend among the data was for higher populations to occur at the Kluver West and Kluver North plots. This could very likely be due to the predominance of the bunch grass *Stipa* sp. at these two sites.

Aboveground arthropod population trends across the growing seasons tended to follow the patterns of aboveground standing live vegetation, i.e., peaking in mid summer and declining toward fall. Nearly all taxonomic and trophic groups tested showed significant ($P < 0.05$) population changes within one or both seasons, most of which had their highest populations near the July sampling. A notable exception is the total arthropods and its major constituent, the Orthoptera, which showed peak populations in the late summer of 1975. The data for these two groups in 1975 are presented in Figures 2.5 and 2.6. The same trend is seen for the trophic group plant tissue feeders. Numerous significant date by treatment interactions ($P \leq 0.05$) are indicative that the observed trends do not necessarily apply equally to all plots. Some of these interactions are presented in Figures 2.7, 2.8, 2.9, and 2.10. Generally, the Kluver East plot has the least change in biomass of various arthropod groups across the growing seasons.

Because of the technical problems encountered in sampling the soil macroarthropods, population changes across the seasons are very difficult to interpret with even greater complications arising due to significant interactions of treatment and date (Tables 2.14 and 2.15). Generally the soil arthropods were composed largely of Homoptera-Cicadidae (root sap feeders) and Coleoptera-Carabidae (largely predatory) and Elateridae (root chewing wire worms). The Cicadids were sampled as mature, emerging nymphs which are generally missed by shallow soil sampling because they normally spend multiple seasons at 40-60 cm depth feeding on root sap and move to the soil surface just prior to emergence to the adult stage. Cicadids were taken at all but the Kluver West plot.

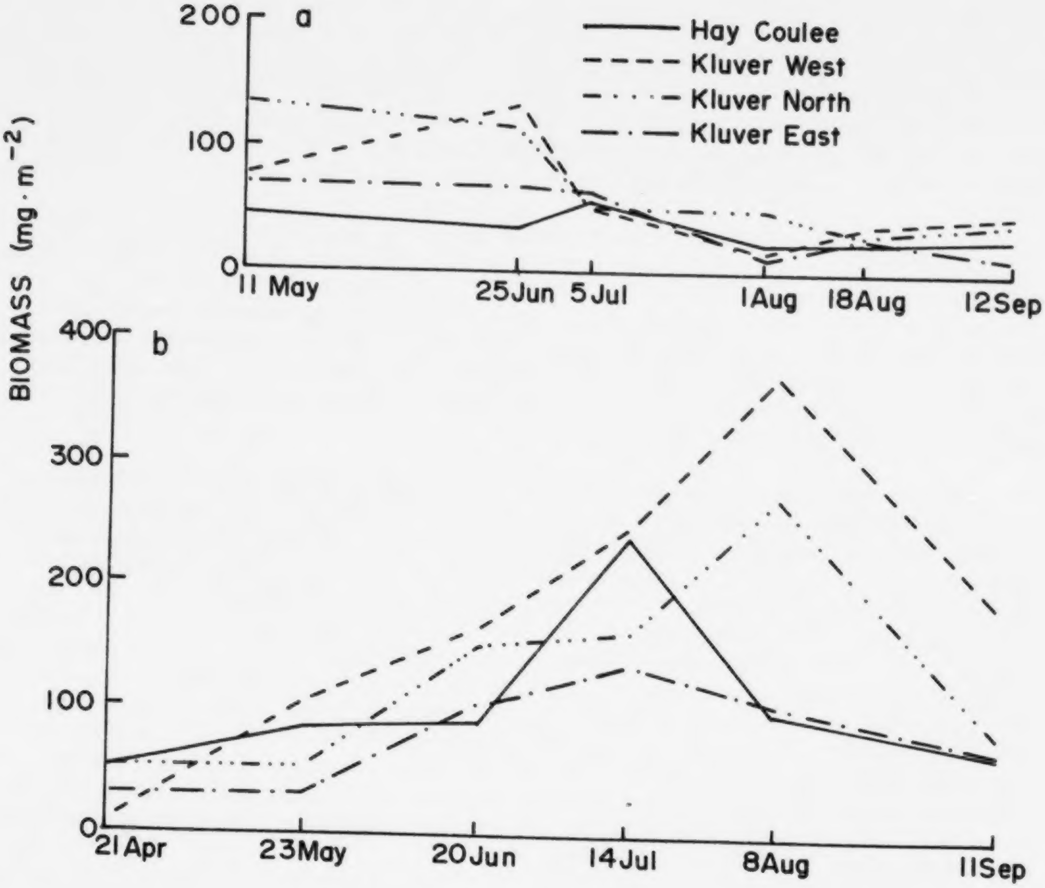


Figure 2.5. Time traces for total aboveground arthropod biomass for 1974 (a) and 1975 (b) for four field plots at Colstrip, Montana.

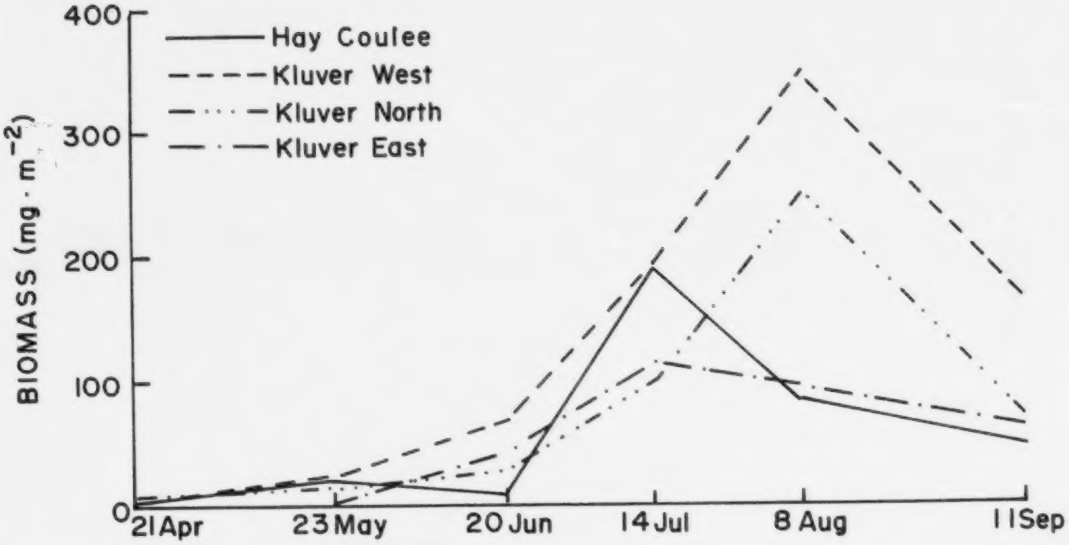


Figure 2.6. Time traces of Orthoptera biomass for four field plots for 1975 at Colstrip, Montana.

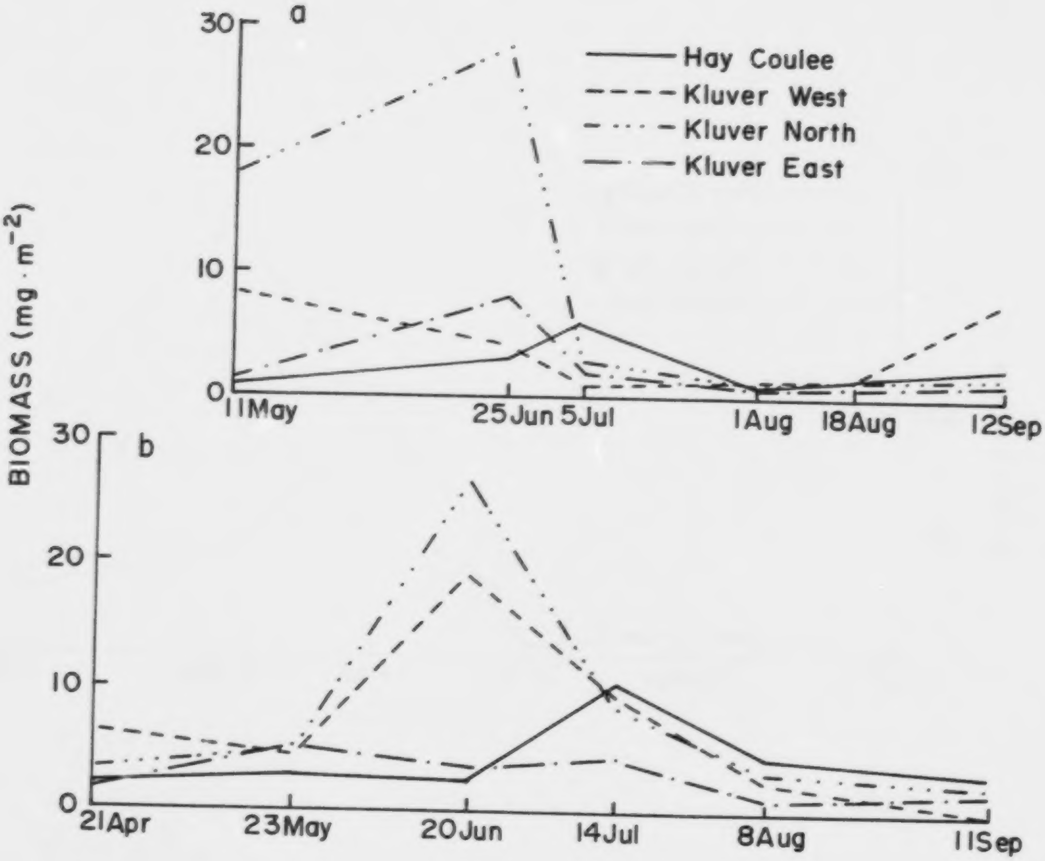


Figure 2.7. Time traces for Hemiptera biomass for 1974 (a) and 1975 (b) on four field plots at Colstrip, Montana.

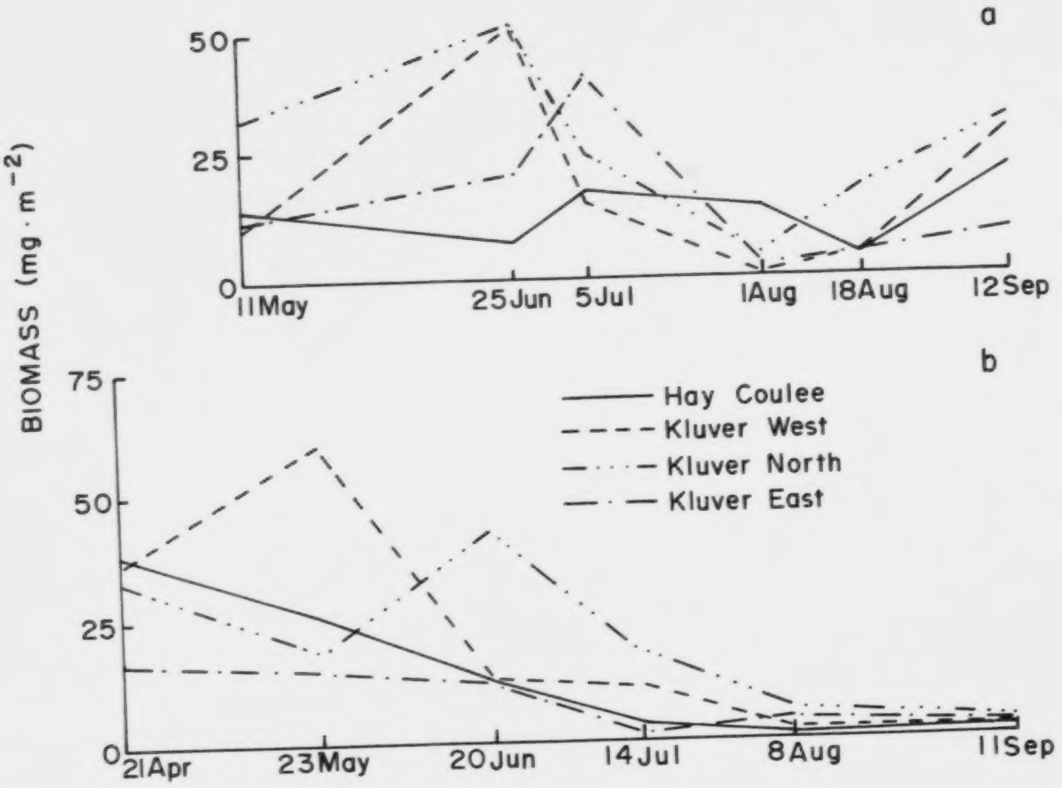


Figure 2.8. Time traces for Coleoptera biomass for 1974 (a) and 1975 (b) on four field plots at Colstrip, Montana.

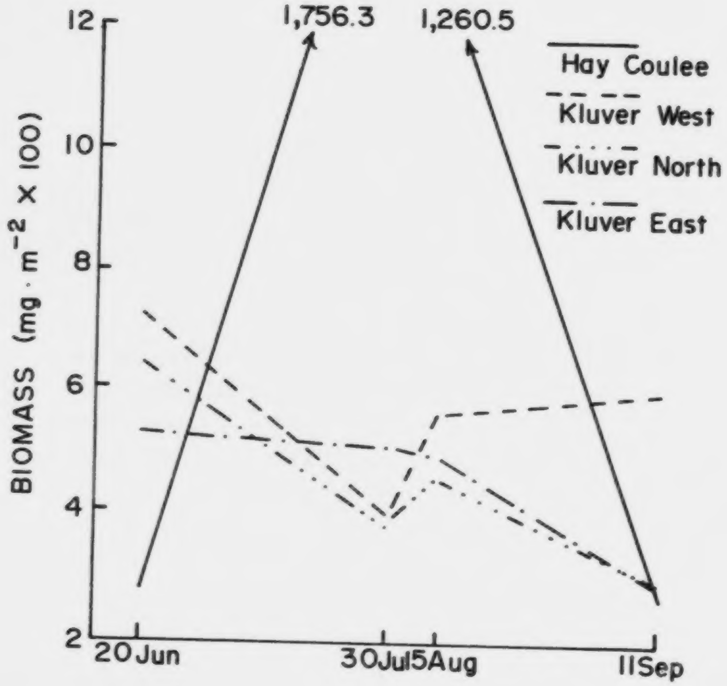


Figure 2.9. Time traces of total soil macroarthropod biomass for four field plots at Colstrip, Montana in 1975.

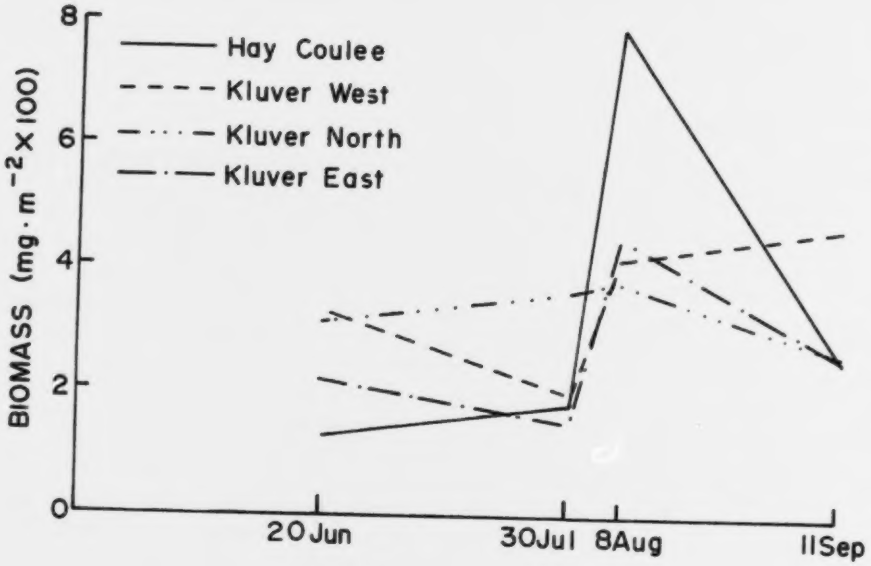


Figure 2.10. Time traces for Coleoptera (soil macroarthropod) biomass for four field plots at Colstrip, Montana in 1975.

The soil microarthropods generally showed a consistent population trend of being high in the spring and declining as the season progresses (Tables 2.16 and 2.17). In 1974 the population showed a resurgence in early fall. These trends are quite closely atuned to soil water dynamics for the seasons. Nearly all groups tested showed significant population changes ($P \leq 0.05$) in one or both seasons and essentially all followed the above trend of high spring and low summer population levels. Only four groups showed significant date × treatment interactions and these data are presented in Figures 2.11, 2.12, 2.13, and 2.14.

For all three types of arthropod data, summaries consisting of means and standard errors have been calculated for all sample dates in both years. The summaries are at the order, suborder, family, and trophic group levels and are done by replicate with treatment summaries. These data are stored at the Natural Resource Ecology Laboratory, Colorado State University, Fort Collins. Anyone interested in any particular facet of these data should contact either Dr. Jerrold L. Dodd or John Leetham. The data are much too voluminous to include here.

Summary and Conclusions

The task of characterizing in depth the complete arthropod fauna of any given ecosystem type is a formidable one and becomes more complex the larger the defined ecosystem type. Because of the immense variability of types and function of the arthropods, it is indeed difficult to devise techniques with which to efficiently census the fauna of a given ecosystem type. The objective in this study was to utilize some techniques developed during the US/IBP Grassland Biome studies to census the arthropod fauna of a northern mixed-grass prairie ecosystem located near the site of a major coal-fired power plant. The censusing was to establish a set of baseline data which may be utilized at a later date to evaluate the long-term effects of the atmospheric pollutants generated by the power plant. Because of the design of the censusing program, only large-scale faunal changes will be detected when return sampling is done. Small-scale, subtle changes may not be detected, or at least not until they have had time to effect larger ramifications in the ecosystem. Nevertheless, the data collected here can provide a reasonably good baseline from which to work.

General characteristics of the arthropod fauna show that an overwhelming majority of the arthropod biomass and numbers occur in the soil (Figure 2.1, Table 2.8). This is not unexpected considering the comparatively xeric conditions of the area and the fact that a majority of the herbage biomass is plant roots. This situation is common to the drier grassland types. A majority of the soil arthropod biomass is made up of Coleoptera and Homoptera immatures (Figure 2.1). However, from a number's view point, the soil acarines are by far the dominant group. The variability of the arthropod fauna within a grassland ecosystem type is exemplified by the grasshopper populations at the four field sites. Slight variations in the controlling environmental factors can result in substantial population variations. Thus the substantially higher *Melanoplus* populations at the Kluver West plot may be due in part to the sandier soil of the area. Sandy loam soils have been

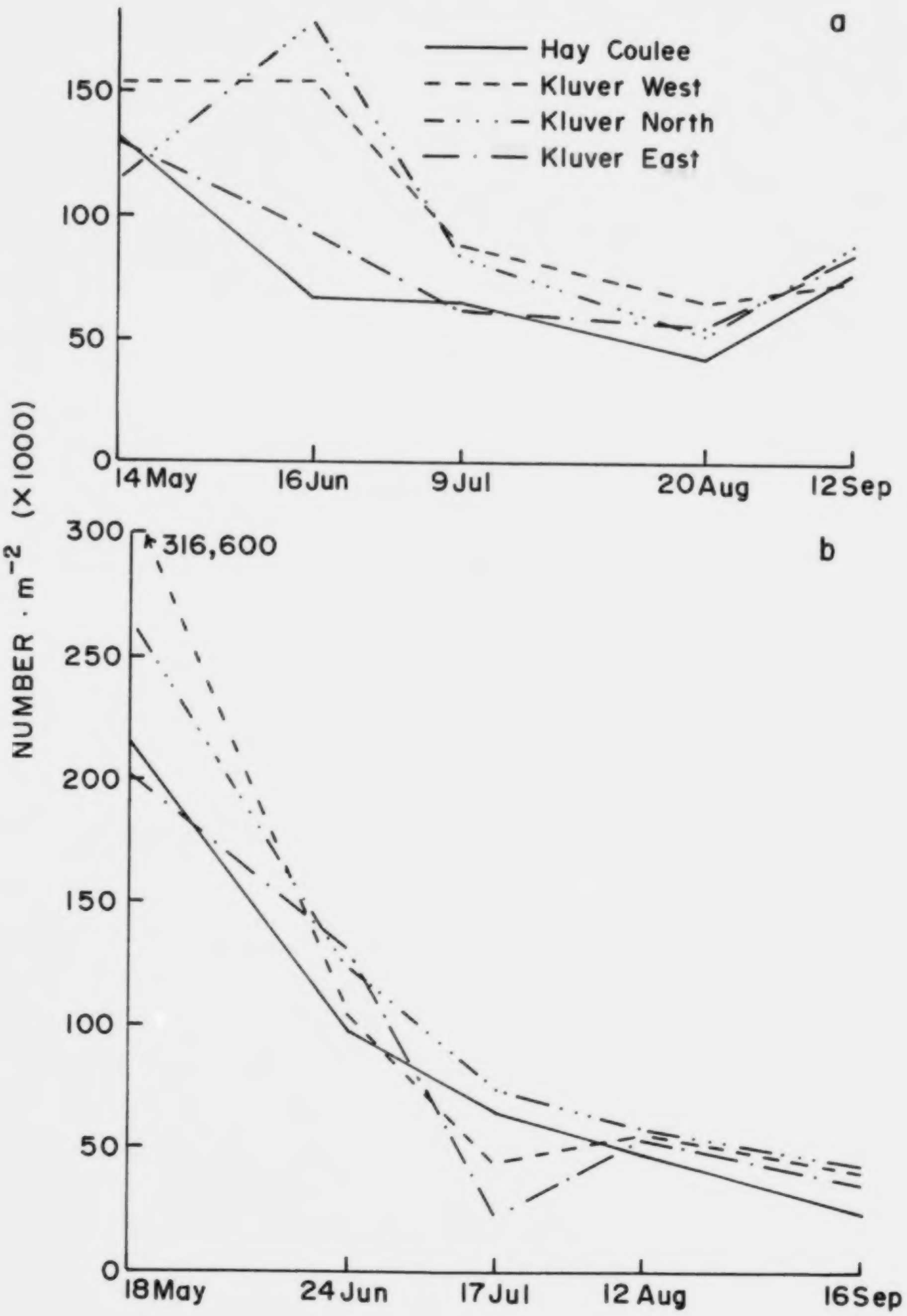


Figure 2.11. Time traces for total soil microarthropod biomass for 1974 (a) and 1975 (b) on four field plots at Colstrip, Montana.

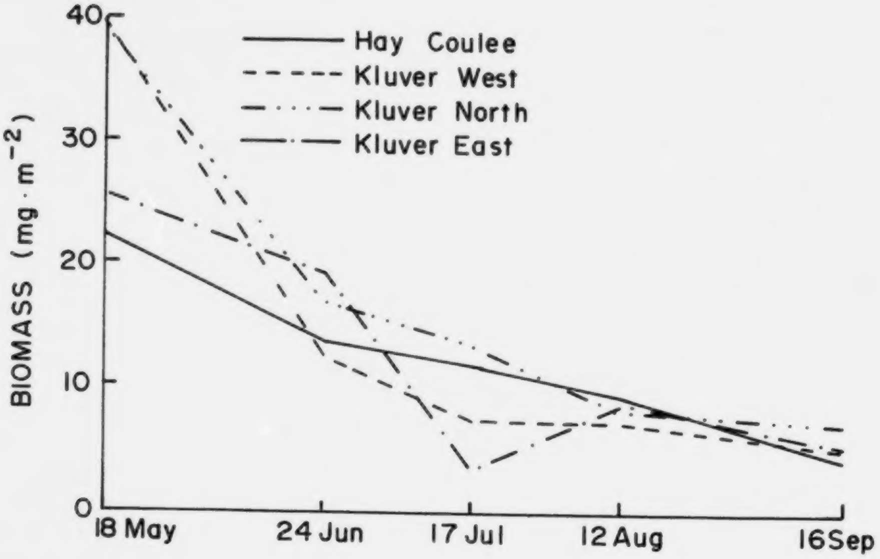


Figure 2.12. Time traces for total biomass of acarine suborder Prostigmata on four field plots in 1975 at Colstrip, Montana.

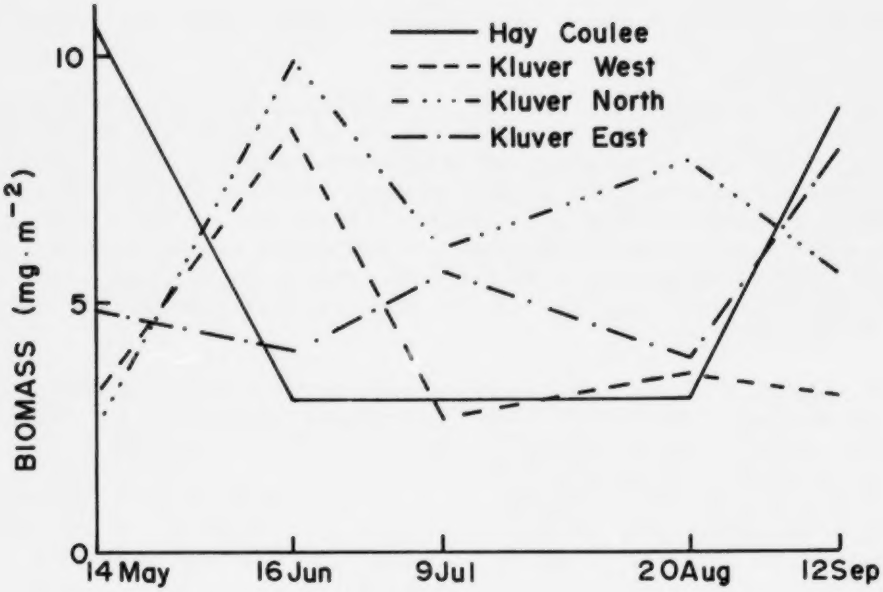


Figure 2.13. Time traces for total biomass of the acarine suborder Mesostigmata on four field plots in 1974 at Colstrip, Montana.

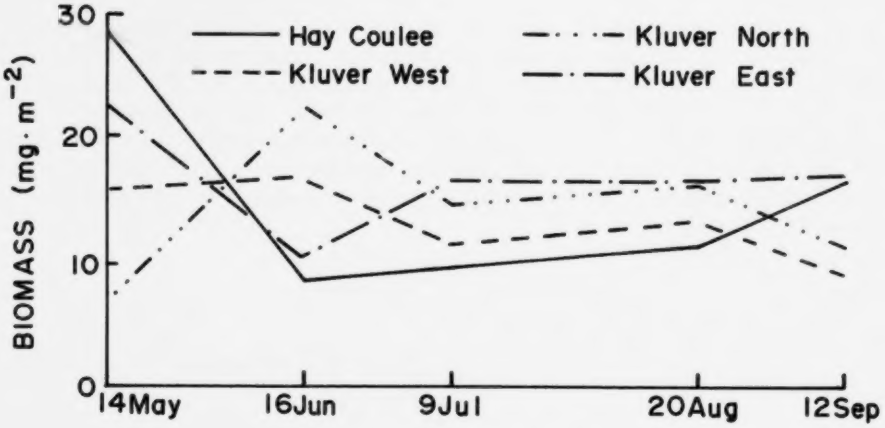


Figure 2.14. Time traces for total biomass of the soil microarthropod trophic group Predator in 1974 on four field plots at Colstrip, Montana.

shown to be preferred by *Melanoplus* species (Isely, 1938) for oviposition sites.

Probably the foremost criterion for utilizing all four field sites for future monitoring is how uniform they are in both floral and faunal aspects. Generally, there are few significant differences between two or more plots for the various taxonomic and trophic groups, although the actual data show substantial variability from plot to plot. Based on our data, the four field plots are good replicates for most of the arthropod groups present. Of course for certain groups such as the grasshoppers where significant differences do occur, the specific outlier plot or plots should not be used for future reference.

The trophic structure of the arthropod fauna is variable among the three divisions sampled (Figure 2.2). The aboveground arthropods are largely herbivores in some way or another. The soil macroarthropods are also largely herbivores; however, there are more predators than above ground. The soil microarthropods are quite different in that herbivores rank third behind fungivores and predators. Herbivory is much less important to the tiny microarthropods, primarily acarines, than the larger insects. They have been able to exploit the large supply of fungal material in the soil and litter zones.

There is a very substantial difference between the season population dynamics of the aboveground arthropods and the soil microarthropods. The aboveground types show population trends similar to the aboveground herbage, *i.e.*, increasing to a mid-season high then declining into winter. This is expected since most of the arthropods are herbivores. By contrast, the soil microarthropods show early spring population highs with declines through the summer and possibly a rebound in fall. This trend is basically the same as the expected soil water cycle, *i.e.*, wetter from spring and early summer rains, drying down in late summer, then rewetting from fall precipitation. Soil macroarthropod trends could not be depicted from our data. The actual trends may be very complicated due to variations in emergence patterns among species. Since many of the macroarthropods are immatures of various insects that also would be classed as aboveground arthropods in the adult stage, their population trends might be coordinated with the availability of food resources for the adult. Thus, a given species may show peak numbers of immatures in the soil late in the growing season when soil water is low but then present high numbers of emerging adults since their primary aboveground food source is then available.

REFERENCES

Isely, F. B. 1938. The Relation of Texas Acrididae to Plants and Soils. Ecol. Monogr., 8:551-604.

Lauenroth, W. K., J. L. Dodd, R. K. Heitschmidt, and R. G. Woodmansee. 1975. Biomass Dynamics and Primary Production in Mixed Prairie Grasslands in Southeastern Montana: Baseline Data for Air Pollution Studies. In: Proceedings of the Fort Union Coal Field Symposium, W. Clark, ed. Eastern Montana College, Billings, Montana. pp. 559-578.

Lauenroth, W. K., J. L. Dodd, R. K. Heitschmidt, and J. W. Leetham. 1978. Effects of SO_2 and Other Coal-firing Plant Emissions on Producer, Invertebrate Consumer, and Decomposer Structure and Function in the Vicinity of Colstrip, Montana. In: The Bioenvironmental Impact of a Coal-fired Power Plant, E. M. Preston and R. A. Lewis, eds. 3rd Interim Rep. EPA-600/3-78-021, U.S. Environmental Protection Agency. Corvallis, Oregon. pp. 13-40.

Leetham, J. W. 1975. A Summary of Field Collecting and Laboratory Processing Equipment and Procedures for Sampling Arthropods at Pawnee Site. US/IBP Grassland Biome Tech. Rep. No. 284. Colorado State University, Fort Collins, Colorado. 49 pp.

McDaniel, B. 1971. Studies of Populations of Adults and Immature Insects and Mites from Two Treatments at Cottonwood, South Dakota. US/IBP Grassland Biome Tech. Rep. No. 112. Colorado State University, Fort Collins, Colorado. 99 pp.

Merchant, V. A., and D. A. Crossley. 1970. An Inexpensive High Efficiency Tullgren Extractor for Soil Microarthropods. J. Ga. Entomol. Soc., 5:83-87.

Riegert, P. W., and J. L. Varley. 1972. Aboveground Invertebrates. I. Population Dynamics. Canadian/IBP Grassland Biome Tech. Rep. No. 6. University of Saskatchewan, Saskatoon, Saskatchewan, Canada. 45 pp.

Riegert, P. W., J. L. Varley, and J. R. Willard. 1974. Aboveground Invertebrates: V. A Summary of Populations, Biomass and Energy Flow. Canadian/IBP Grassland Biome Tech. Rep. No. 67. University of Saskatchewan, Saskatoon, Saskatchewan, Canada. 28 pp.

Taylor, J. E., W. C. Leininger, and R. J. Fuchs. 1976. Site Descriptions and Effects of Coal-fired Power Plant Emissions on Plant Community Structure. In: The Bioenvironmental Effects of a Coal-fired Power Plant, R. A. Lewis, N. R. Glass, and A. S. Lefohn, eds. 2nd Interim Rep. EPA-600/3-76-013, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 11-39.

Willard, J. R. 1974. Soil Invertebrates: VIII. A Summary of Populations and Biomass. Canadian/IBP Grassland Biome Tech. Rep. No. 56. University of Saskatchewan, Saskatoon, Saskatchewan, Canada. 110 pp.

APPENDIX

TABLE 2.1. PHYSICAL CHARACTERISTICS OF COLSTRIP STUDY SITE SOILS

Sample number	Horizon	Depth (cm)	Gravel (%)	Sand (%)	Silt (%)	Clay (%)	Texture*	Bulk density
			Hay Coulee, North Pit					
228P	A1	0-12	2.76	23	40	37	CL	1.18
400P	Dupl.	0-12	2.76	24	40	36	CL	1.18
234P	B2	12-22	2.35	18	46	36	SiCL	
401P	C1	22-60	3.06	15	44	41	SiC	
402P	Dupl.	22-60	3.06	15	45	40	SiC	
229P	C2	60-98	2.12	28	48	24	L	
230P	C3	98-152	2.02	26	45	29	CL	
			Hay Coulee, South Pit					
270P	A1	0-12	1.87	40	40	20	L	1.20
271P	B21	12-20	1.64	52	15	33	SCL	1.33
404P	Dupl.	12-20	1.64	49	19	32	SCL	1.33
272P	B22	20-33	1.47	58	16	26	SCL	1.29
273P	B3	33-43	2.17	63	14	23	SCL	1.59
274P	C1	43-90	1.98	63	15	22	SCL	
275P	C2	90-152	1.66	45	28	27	SCL	
			Kluver West, East Pit					
262P	A1	0-10	8.08	52	24	24	SCL	1.25
264P	B2	10-20	4.14	43	26	31	CL	1.37
403P	Dupl.	10-20	4.14	41	29	30	CL	1.37
265P	B3ca	20-33	7.34	40	32	28	CL	1.42
267P	C1ca	33-62.5	4.31	54	22	24	SCL	1.49
268P	IIC1	62.5-85	59.37	76	8	16	SL	
269P	IIC2	85+	4.22	62	20	18	SL	
			Kluver West, West Pit					
311P	A1	0-10	7.84	54	24	22	SCL	1.28
312P	B2	10-31	4.19	52	21	27	SCL	1.40
313P	B3	31-40	29.98	59	19	22	SCL	1.59
314P	C1ca	40-118	5.97	55	22	23	SCL	
315P	C2ca	118-156	15.31	72	13	15	SL	

TABLE 2.1. CONTINUED.

Sample number	Horizon	Depth (cm)	Gravel (%)	Sand (%)	Silt (%)	Clay (%)	Texture*	Bulk density
Kluver North, East Pit								
245P	A1	0-10	2.60	61	15	24	SCL	1.25
246P	B2	10-30	4.62	58	18	24	SCL	1.44
247P	B3ca	30-48	0.33	48	23	29	SCL	1.54
248P	C1	48-64	3.83	45	26	29	SCL	1.57
249P	C2	64-90	0.29	41	27	32	CL	
250P	C3	90-152	0.13	44	26	30	CL	
Kluver North, West Pit								
296P	A1	0-13	1.59	58	21	21	SCL	1.11
297P	B1	13-24	0.37	58	18	24	SCL	1.38
298P	B2	24-40	1.44	60	16	24	SCL	1.51
299P	B3	40-50	1.53	58	18	24	SCL	1.58
300P	C1	50-84	0.73	55	16	29	SCL	1.54
301P	C2	84-114	0.68	56	16	28	SCL	
302P	C3	114-152	0.00	54	20	26	SCL	
Kluver East, South Pit								
283P	A1	0-10	66.39	35	31	34	CL	1.29
284P	B2ca	10-20	3.02	34	34	32	CL	1.38
285P	B3ca	20-28	0.79	34	34	32	CL	1.59
286P	C1ca	28-46	1.38	35	33	32	CL	1.59
287P	C2ca	46-108	0.40	64	13	23	SCL	
288P	C3	108-152	31.47	61	14	25	SCL	

*C = clay, L = loam, Si = silt, S = sand.

TABLE 2.2. WATER-HOLDING CAPACITY (% WT) OF COLSTRIP STUDY SITE SOILS

Sample number	Horizon	Depth (cm)	0 bar		.33 bar		1 bar		15 bar	
			Hay Coulee, North Pit							
228P	A1	0-12	64.46	62.70	25.11	23.10	19.49	19.38	9.79	9.90
400P	Dupl.	0-12								
234P	B2	12-22	43.23	57.55	23.34	22.68	17.42	17.24	9.92	9.53
401P	C1	22-60								
402P	Dupl.	22-60								
229P	C2	60-98	51.02	54.21	20.16	20.06	14.05	13.93	6.15	6.63
230P	C3	98-152	47.32	47.94	21.63	20.93	13.88	13.07	6.63	6.75
			Hay Coulee, South Pit							
270P	A1	0-12	42.87	48.35	17.89	19.09	14.31	14.42	7.70	7.82
271P	B21	12-20	53.70	53.08	16.92	16.45	11.57	14.20	9.19	8.78
404P	Dupl.	12-20								
272P	B22	20-33	49.73	49.71	15.78	14.89	12.53	12.69	7.87	7.22
273P	B3	33-43	44.46	41.99	14.35	14.40	11.19	10.85	5.87	5.96
274P	C1	43-90	41.94	41.90	12.83	12.90	10.08	10.32	5.84	5.40
275P	C2	90-152	44.07	44.62	17.42	17.44	13.93	13.63	6.93	6.39
			Kluver West, East Pit							
262P	A1	0-10	44.81	45.76	13.96	14.96	12.04	12.59	6.49	6.44
264P	B2	10-20	54.28	52.06	18.23	18.36	14.60	12.43	8.82	9.10
403P	Dupl.	10-20								
265P	B3ca	20-33	50.64	52.17	20.26	18.87	14.17	14.27	7.10	7.26
267P	C1ca	33-62.5	43.70	43.85	15.77	16.06	13.01	13.34	5.66	5.72
268P	IIC1	62.5-85	34.03	35.66	9.09	9.94	7.46	7.32	3.96	4.55
269P	IIC2	85+	58.41	26.74	12.91	13.68	9.36	7.41	4.65	4.72

TABLE 2.2. CONTINUED.

Sample number	Horizon	Depth (cm)	0 bar		.33 bar		1 bar		15 bar	
					Kluver West, West Pit					
311P	A1	0-10	40.86	42.76	15.20	14.36	10.64	10.61	4.86	5.02
312P	B2	10-31	48.02	45.99	13.45	11.96	11.43	11.47	6.93	7.60
313P	B3	31-40	58.81	42.77	23.56	19.31	10.26	9.92	5.87	5.29
314P	C1ca	40-118	42.44	41.34	14.62	14.90	11.61	11.61	5.32	5.31
315P	C2ca	118-156	34.82	33.81	10.56	12.06	7.41	7.57	3.65	3.49
					Kluver North, East Pit					
245P	A1	0-10	46.14	44.37	14.68	13.79	11.35	11.45	7.43	7.15
246P	B2	10-30	51.03	50.75	15.95	15.41	12.59	12.12	7.25	7.15
247P	B3ca	30-48	49.91	51.08	20.15	19.56	14.75	14.36	8.17	8.13
248P	C1	48-64	49.54	55.18	17.39	17.75	14.95	15.46	7.56	7.38
249P	C2	64-90	52.48	53.76	21.64	20.06	16.39	16.16	8.78	8.72
250P	C3	90-152	43.68	60.64	18.00	17.77	14.29	15.74	7.58	7.89
					Kluver North, West Pit					
296P	A1	0-13	43.19	41.92	13.60	12.33	10.45	10.21	6.10	5.67
297P	B1	13-24	57.02	51.71	13.09	16.01	11.06	11.18	6.50	6.48
298P	B2	24.40	48.35	50.81	11.27	13.04	11.06	10.74	6.81	6.20
299P	B3	40-50	48.48	49.34						
300P	C1	50-84	48.70	50.36	15.68	15.40	13.08	13.06	7.05	7.05
301P	C2	84-114	47.05	45.83	14.80	15.75	11.97	12.56	6.58	6.60
302P	C3	114-152	43.48	44.12	12.76	13.38	13.60	11.02	4.83	5.28

TABLE 2.2. CONTINUED.

Sample number	Horizon	Depth (cm)	0 bar		.33 bar		1 bar		15 bar	
			Kluver East, South Pit							
283P	A1	0-10	57.79	52.23	18.00	18.15	15.31	15.00	8.76	8.09
284P	B2ca	10-20	53.77	55.16	22.38	20.37	15.83	15.59	8.30	8.86
285P	B3ca	20-28	50.11	31.00	22.55	23.44	16.24	16.79	8.68	8.19
286P	C1ca	28-46	54.42	54.37	20.73	21.04	15.63	15.59	8.48	8.02
287P	C2ca	46-108	38.08	38.24	12.30	12.55	9.31	9.27	4.72	4.55
288P	C3	108-152	34.44	36.38	11.87	12.25	9.55	10.24	4.79	4.59

TABLE 2.3. CHEMICAL CONSTITUENTS OF COLSTRIP STUDY SITE SOILS

Sample number	Horizon	Depth (cm)	Organic matter (%)	pH	Lime (%)	N (%)	Total P (%)	Inorganic P (%)	Bicarbonate P (%)
				Hay Coulee, North Pit					
228P	A1	0-12	1.4	7.4	5.0	0.108	0.058	0.044	1
400P	Dupl.	0-12	1.5	7.4	4.6	0.105	0.041	0.030	2
234P	B2	12-22	1.3	7.4	11.8	0.110	0.060	0.044	1
401P	C1	22-60	0.9	8.3	23.6	0.068	0.045	0.036	1
402P	Dupl.	22-60	0.5	8.5	22.6	0.037	0.041	0.036	1
229P	C2	60-98	0.3	8.4	15.8	0.028	0.050	0.045	1
230P	C3	98-152	0.3	8.9	15.2	0.018	0.050	0.044	5
				Hay Coulee, South Pit					
270P	A1	0-12	1.5	7.1	0.0	0.108	0.045	0.030	2
271P	B21	12-20	1.0	7.1	0.0	0.081	0.042	0.030	2
404P	Dupl.	12-20	1.1	7.0	0.0	0.082	0.041	0.026	2
272P	B22	20-33	0.9	9.9	4.2	0.075	0.045	0.033	2
273P	B3	33-43	0.9	8.0	11.4	0.062	0.042	0.035	1
274P	C1	43-90	0.7	8.1	14.8	0.055	0.040	0.034	1
275P	C2	90-152	0.3	8.4	15.6	0.021	0.034	0.030	2
				Kluver West, East Pit					
262P	A1	0-10	1.6	6.7	0.0	0.105	0.050	0.030	5
264P	B2	10-20	1.3	7.5	1.8	0.101	0.050	0.035	1
403P	Dupl.	10-20	1.6	7.6	2.4	0.100	0.045	0.031	1
265P	B3ca	20-33	1.2	8.0	14.2	0.090	0.056	0.039	1
267P	C1ca	33-62.5	0.7	8.2	12.8	0.052	0.050	0.037	1
268P	IIC1	62.5-85	0.4	8.4	11.2	0.025	0.039	0.032	1
269P	IIC2	85+	0.3	8.5	10.8	0.016	0.036	0.032	1

TABLE 2.3. CONTINUED.

Sample number	Horizon	Depth (cm)	Organic matter (%)	pH	Lime (%)	N (%)	Total P (%)	Inorganic P (%)	Bicarbonate P (%)
				Kluver West, West Pit					
311P	A1	0-10	1.3	7.0	0.0	0.089	0.042	0.028	2
312P	B2	10-31	1.2	7.0	0.0	0.073	0.040	0.027	1
313P	B3	31-40	0.8	8.1	12.6	0.064	0.045	0.032	1
314P	C1ca	40-118	0.5	8.5	15.6	0.038	0.038	0.031	1
315P	C2ca	118-156	0.2	8.6	10.0	0.013	0.033	0.031	1
				Kluver North, East Pit					
245P	A1	0-10	1.2	6.8	0.0	0.085	0.040	0.030	2
246P	B2	10-30	1.1	7.7	0.8	0.081	0.043	0.033	2
247P	B3ca	30-48	1.0	8.2	10.8	0.059	0.052	0.036	1
248P	C1	48-64	0.8	9.3	11.4	0.052	0.046	0.036	1
249P	C2	64-90	1.0	8.5	12.6	0.047	0.043	0.036	1
250P	C3	90-152	0.6	8.5	7.2	0.031	0.045	0.041	7
				Kluver North, West Pit					
296P	A1	0-13	1.5	6.8	0.0	0.105	0.042	0.032	3
297P	B1	13-24	0.9	7.0	0.0	0.070	0.038	0.031	4
298P	B2	24-40	1.0	7.6	2.0	0.074	0.045	0.028	1
299P	B3	40-50	1.0	7.8	1.6	0.083	0.047	0.032	2
300P	C1	50-84	0.7	8.5	13.2	0.046	0.040	0.032	3
301P	C2	84-114	0.4	8.5	14.0	0.027	0.038	0.033	1
302P	C3	114-152	0.4	8.4	17.8	0.021	0.040	0.036	3

TABLE 2.3. CONTINUED.

Sample number	Horizon	Depth (cm)	Organic matter (%)	pH	Lime (%)	N (%)	Total P (%)	Inorganic P (%)	Bicarbonate P (%)
			Kluver East, South Pit						
283P	A1	0-10	1.8	7.5	2.2	0.125	0.056	0.032	2
284P	B2ca	10-20	1.3	8.0	13.0	0.101	0.043	0.032	1
285P	B3ca	20-28	1.2	7.9	17.0	0.088	0.040	0.030	1
286P	C1ca	28-46	1.0	8.2	18.4	0.085	0.040	0.029	1
287P	C2ca	46-108	0.4	8.5	14.0	0.020	0.036	0.032	3
288P	C3	108-152	0.3	8.3	12.0	0.014	0.034	0.030	4

TABLE 2.4. EXCHANGEABLE IONS OF COLSTRIP STUDY SITE SOILS (MEQ · 100 G^{-1} SOIL)

Sample number	Horizon	Depth (cm)	SO_4	CEC	Ca	Mg	Na	K	K (ppm)
Hay Coulee, North Pit									
228P	A1	0-12	<0.1	27.7	--	--	0.2	0.6	230
400P	Dupl.	0-12	<0.1	16.8	--	--	0.2	0.6	240
234P	B2	12-22	<0.1	28.6	--	--	0.1	0.3	110
401P	C1	22-60	1.2	13.0	--	--	0.8	0.2	65
402P	Dupl.	22-60	1.9	10.5	--	--	1.4	0.1	55
229P	C2	60-98	36.4	13.8	--	--	3.2	0.1	40
230P	C3	98-152	71.6	14.1	--	--	4.4	0.1	55
Hay Coulee, South Pit									
270P	A1	0-12	0.2	23.3	16.0	5.7	<0.1	0.6	225
271P	B21	12-20	0.1	28.2	19.0	7.4	<0.1	0.4	140
404P	Dupl.	12-20	<0.1	17.0	10.8	5.3	0.1	0.4	170
272P	B22	20-33	<0.1	21.4	--	--	<0.1	0.2	90
273P	B3	33-43	<0.1	15.5	--	--	<0.1	0.1	55
274P	C1	43-90	0.1	12.4	--	--	<0.1	0.1	40
275P	C2	90-152	0.3	14.7	--	--	0.3	0.1	55
Kluver West, East Pit									
262P	A1	0-10	0.4	21.8	14.3	4.6	0.1	0.6	215
264P	B2	10-20	0.1	26.7	--	--	0.1	0.6	215
403P	Dupl.	10-20	<0.1	15.1	--	--	0.1	0.6	235
265P	B3ca	20-33	0.1	20.8	--	--	0.1	0.3	105
267P	C1ca	33-62.5	<0.1	15.5	--	--	0.1	0.2	75
268P	IIC1	62.5-85	<0.1	9.1	--	--	0.1	0.1	35
269P	IIC2	85+	0.2	10.0	--	--	0.1	0.1	50

TABLE 2.4. CONTINUED.

Sample number	Horizon	Depth (cm)	SO_4	CEC	Ca	Mg	Na	K	K (ppm)
Kluver West, West Pit									
311P	A1	0-10	0.1	9.8	6.6	2.3	<0.1	0.4	175
312P	B2	10-31	1.9	12.8	10.2	2.3	<0.1	0.4	155
313P	B3	31-40	<0.1	8.8	--	--	<0.1	0.2	60
314P	C1ca	40-118	<0.1	7.8	--	--	0.1	0.1	50
315P	C2ca	118-156	3.9	5.0	--	--	0.5	0.1	50
Kluver North, East Pit									
245P	A1	0-10	0.4	24.4	15.3	5.2	0.1	0.4	175
246P	B2	10-30	<0.1	25.6	--	--	0.1	0.2	90
247P	B3ca	30-48	<0.1	24.7	--	--	0.1	0.2	65
248P	C1	48-64	<0.1	24.5	--	--	0.1	0.2	75
249P	C2	64-90	<0.1	29.6	--	--	0.3	0.2	65
250P	C3	90-152	1.7	24.7	--	--	1.0	0.2	80
Kluver North, West Pit									
296P	A1	0-13	0.2	18.1	13.0	3.6	<0.1	0.4	160
297P	B1	13-24	0.2	20.4	14.3	4.7	<0.1	0.5	180
298P	B2	24-40	<0.1	11.5	--	--	<0.1	0.3	115
299P	B3	40-50	<0.1	12.3	--	--	<0.1	0.3	110
300P	C1	50-84	<0.1	10.4	--	--	<0.1	0.2	65
301P	C2	84-114	<0.1	10.2	--	--	0.1	0.2	65
302P	C3	114-152	0.3	7.1	--	--	0.1	0.1	55

TABLE 2.4. CONTINUED.

Sample number	Horizon	Depth (cm)	SO_4	CEC	Ca	Mg	Na	K	K (ppm)
				Kluver East, South Pit					
283P	A1	0-10	<0.1	25.5	--	--	<0.1	0.6	235
284P	B2ca	10-20	<0.1	20.6	--	--	<0.1	0.2	95
285P	B3ca	20-28	<0.1	18.6	--	--	0.1	0.1	50
286P	C1ca	28-46	<0.1	15.4	--	--	0.1	0.1	45
287P	C2ca	46-108	0.3	10.0	--	--	0.1	0.1	35
288P	C3	108-152	13.5	10.6	--	--	0.8	0.1	40

TABLE 2.5. PRECIPITATION AMOUNTS AT COLSTRIP VICINITY STUDY SITES, 1976 *

Date	Hay Coulee	Kluver East	Kluver North	Kluver West
4-15 †				
4-21	----§	----	0.56	----
4-23	0.84	----	----	----
4-27	----	0.54	----	----
4-28	0.64	----	0.60	0.66
4-29	0.24	----	----	----
5-05	1.01	1.80	1.78	1.58
5-06	0.02	----	----	----
5-11	0.20	----	----	----
5-12	0.63	----	----	----
5-14	0.23	0.66	0.80	0.83
5-17	----	0.14	0.25	0.32
5-24	0.03	0.00	0.07	0.20
5-26	0.18	----	----	----
5-29	0.34	----	----	----
5-31	----	0.22	0.36	0.30
6-02	0.03	----	----	----
6-03	----	0.10	0.00	0.01
6-05	0.16	----	----	----
6-07	0.40	0.68	0.59	----
6-09	0.15	----	----	----
6-11	0.03	----	----	----
6-13	0.60	----	----	----
6-15	1.06	1.57	2.03	2.14
6-17	0.53	----	----	----
6-18	----	0.46	0.46	0.50
6-21	0.46	0.53	0.29	0.23
6-23	0.63	----	----	----
6-24	0.03	0.46	0.70	0.97
6-25	0.20	----	----	----
6-28	----	0.15	0.25	0.21
7-02	0.14	----	----	----
7-05	----	0.10	0.04	0.02
7-13	0.30	----	----	----
7-16	----	0.20	0.17	0.14
7-30	----	0.40	----	----
8-03	0.05	----	----	----

(continued)

TABLE 2.5. (continued)

Date	Hay Coulee	Kluver		
		East	North	West
8-16	----	----	0.07	0.05
8-19	----	0.07	0.00	0.13
8-24	0.04	----	----	----
8-26	----	0.25	0.10	0.10
8-28	0.02	----	----	----
9-03	----	0.02	----	----
9-07	0.36	0.38	0.33	0.30
9-15	0.03	----	----	----
9-20	0.05	----	0.02	0.02
9-27	0.22	0.30	0.22	0.07
10-04	0.06	0.09	0.03	0.02
10-06	0.28	----	----	----
10-07	0.05	0.18	0.17	0.28
10-18	----	≈0.65 ψ	≈0.65 ψ	≈0.65 ψ
10-27	0.35	----	----	----
11-02	0.01	----	----	----
11-18	0.03	----	----	----

[*] Inches accumulated following previous reading

[†] Beginning date of record

[§] Hyphens indicate no reading taken

[ψ] 7" snow

SECTION 3

PLANT COMMUNITY MONITORING

J. E. Taylor and W. C. Leininger

ABSTRACT

Plant community studies have been conducted near Colstrip since 1974. These include estimates of species cover, diversity (based on number and canopy coverage), and phenology. Photographic records, both aerial and ground-level, also are being made. Data are collected within the exclosures established by the EPA and on three contiguous relict knolls along Rosebud Creek. In addition, an aerial photo transect from Colstrip southeast to Greenleaf Creek is periodically flown. Graminoids and lichens are the dominant vegetational components influencing cover, number, and diversity. The abundance of annual grasses varies markedly among sites, and this strongly affects diversity through reduced equitability. Previous grazing history and variations in yearly climatic conditions, which are primarily responsible for the observed differences in plant species composition, have so far masked any pollution effects. Our data suggest a predictive relationship between plant diversity and range condition. Phenology data have been more useful in interpreting aerial photography than in directly monitoring plant community changes. May and June aerial photo missions were flown with fixed wing aircraft in 1977. In August, the Colstrip sites were flown at larger scale from a helicopter. This photography contributes to the long-term baseline/response record. In addition, site mapping is proceeding with these photographic base maps.

INTRODUCTION

This research was begun 15 July 1974 to monitor bioenvironmental effects of coal-fired electric generating plants in southeastern Montana. Our objectives are:

1. To characterize pre-treatment native plant communities in areas likely to be affected by fossil fuel power plants.
2. To develop measurement techniques and monitor changes in plant community structure, diversity, phenology and species composition following air pollution stress.
3. To develop methods which can be used to predict bioenvironmental changes following fossil fuel power generation in other areas.

The data reported in this section cover only our research activities in the vicinity of Colstrip, Montana. In addition to four exclosures established and maintained by the EPA, three relatively pristine knolls are being examined (Figure 3.1.). Previous work and study site descriptions have been summarized by Taylor and Leininger (1977).

MATERIALS AND METHODS

Plant community analysis and photographic monitoring are discussed in detail below.

Plant Community Analysis

Canopy Coverage

Canopy coverage estimates were made on all study sites in either late August or early September, 1977. Sample plots within study areas were located by placing a cord with meter-spaced knots in a random meandering pattern through the sample area. Then 2 x 5 dm plot frames were placed at each knot and canopy coverage estimated using the procedures of Daubenmire (1959). Two sets (lines) of 25 frames each were sampled.

Species Diversity

The diversity index used was the Shannon-Weaver function (Shannon and Weaver, 1949):

$$H' = - \sum_{i=1}^{S} \frac{Ni}{N} \log_2 \frac{Ni}{N}$$

Where H' = Index of diversity
S = number of species
Ni = number of ith species
N = total number of all species

For canopy coverage diversity, percent canopy estimates were used. Canopy coverage and species and individuals per species were recorded for each frame concurrently. Both kinds of data have been analyzed to characterize the species diversity of each study site.

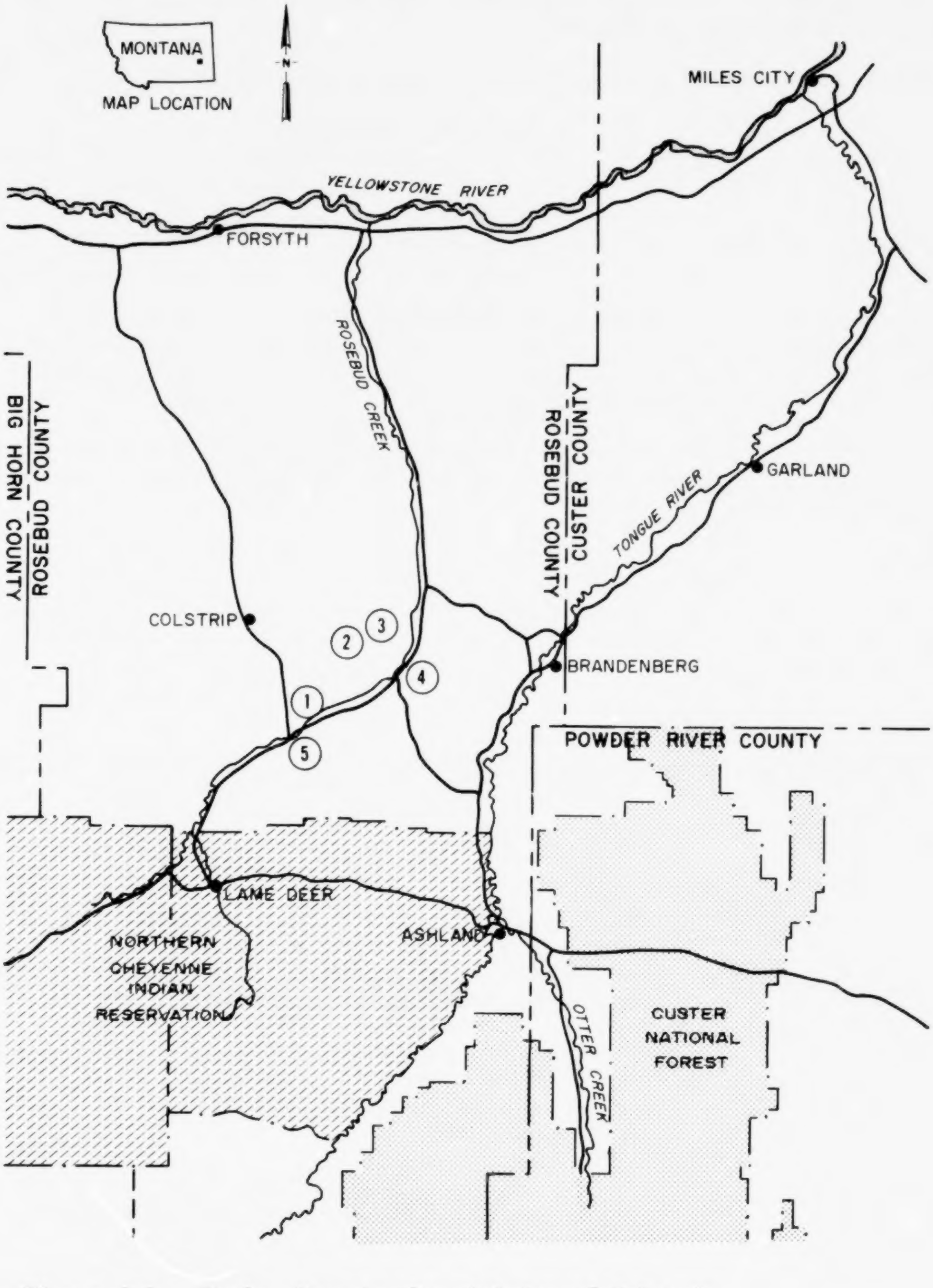

MONTANA
MAP LOCATION
N
MILES CITY
YELLOWSTONE RIVER
FORSYTH
ROSEBUD CREEK
BIG HORN COUNTY
ROSEBUD COUNTY
ROSEBUD COUNTY
CUSTER COUNTY
GARLAND
TONGUE RIVER
COLSTRIP
2
3
4
1
5
BRANDENBERG
POWDER RIVER COUNTY
LAME DEER
ASHLAND
NORTHERN
CHEYENNE
INDIAN
RESERVATION
OTTER CREEK
CUSTER
NATIONAL
FOREST

Figure 3.1. Study sites in the vicinity of Colstrip.
(1= Hay Coulee, 2 = Kluver West, 3 = Kluver North,
4 = Kluver East, 5 = McRae Knolls)

In addition to diversity, these data were used to calculate evenness (equitability) and species richness.

Evenness was calculated with the equation of Pielou (1969).

$$J' = \frac{H'}{\log_2 S}$$

Where J' = Evenness
H' = Shannon-Weaver index
S = total number of species

Species richness is the numerical sum of species which were used to calculate H'.

In order to reduce variances and still be able to calculate standard errors, data were grouped by five consecutive frame sums (cover) or means (number) before H' or J' were calculated. S was the total number of species encountered in each five-frame group.

There were a number of species on the sites which were never encountered in the diversity samples. A list of all species observed is presented as Appendix 3.1.

Plant Phenology

Upon each visit to an experimental site, the modal phenological stage of each identifiable plant species is characterized. The classification system is shown in Table 3.1.

TABLE 3.1. PLANT PHENOLOGY INDEX

Phenology Code	Stage of Growth
1	First growth
2	First leaves fully expanded
3	Active vegetative growth
4	Vegetative growth mostly complete
5	Boot stage, first floral buds
6	Exsertion of grass inflorescences, earliest flowers
7	Reproductive culms fully extended
8	Anthesis, full flowering
9	Fruit developing
10	Fruit ripe
11	Dehiscence
12	Vegetative maturity, summer or winter dormancy, leaf drop, annuals dead

Note: seedlings, basal rosettes, etc., handled in notes; fall greenup recorded as Code 3.

Photographic Studies

Ground Level Photography

Ground level photography provides a detailed record of plant species, phenology, and pathologic signs. This assists in the interpretation of aerial imagery. Also, vertical ground photo plots may be measured and analyzed for cover, number, frequency, pattern, and plant volume. Plant volume, which can be used to estimate biomass, may be obtained by combining canopy coverage and height, the latter measured with a parallax wedge. Plant density and pattern also can be studied from these pictures.

At the Hay Coulee and Kluver sites, two photo plots were established in each exclosure, while one photo plot was placed on each of the three McRae Knolls.

The photo plots are 1 meter square in area, and are marked for relocation. Each is photographed in color and black-and-white film emulsions. Stereoscopic photography is used for ease of plant identification. Most of the plots have also been photographed with infrared color film. More details are given by Taylor *et al.*, 1976.

Aspect photographs are made from vantage points within and overlooking study areas. These are taken with color and infrared color film, the latter to compare with aerial coverage.

For each photo plot an index of species identification has been prepared. The combination of plot photographs and plot indices makes a permanent record of species presence and distribution. Sequential records allow the evaluation of temporal changes.

Aerial Photography

Low level aerial photography gives a more generalized view of plant species and community distribution, pathology, and cover than does ground photography. Further, it yields more detail than higher level imagery and so represents a useful compromise between detail and comprehensiveness of coverage.

Low level aerial imagery is most practical for making detailed vegetation maps, sensing population-level stresses, or any other purposes requiring large scale synoptic views. It also aids in developing interpretations of smaller scale, high level photography (Taylor, 1976).

For our overflights we use a Cessna 182 airplane which can easily handle the required elevation range of 500 to 7,000 feet above the ground. The plane we use is leased from Miles City Aero Services, Miles City, Montana. This aircraft has been modified by the addition of a 30.5 cm diameter belly port, which accepts a special camera mount, designed and manufactured by W. E. Woodcock of Miles City (Woodcock, 1976). The mount supports a Hasselblad EL/M motor driven camera (70 mm format). It is fitted with 50 or 80 mm lenses, depending on the desired photo scale.

In addition to the mounted camera, a second Hasselblad is used as a hand camera for making oblique photographs from the air. This kind of photography supplements the more traditional vertical imagery, in that it is more representative of familiar aspects of scenes for interpretation, display, etc.

In August, 1977 we contracted with Hawkins and Power, Greybull, Wyoming, for a helicopter photo mission over the Colstrip sites. Flying altitudes ranged from 73 to 218 meters, yielding negative scales from 1:900 to 1:2700. Color negative film was exposed in hand-held Hasselblad cameras while the helicopter was slowly traversing the targets. Vertical photography was achieved with a bubble level attached to the camera back.

Color prints of this coverage will be used as base photographs for detailed vegetation mapping during the 1978 growing season.

We use three primary film types, with other materials for special purposes. Our main films are color, color infrared and black-and-white (H&W VTE Pan). Each of these has advantages for particular use.

RESULTS AND DISCUSSION

Plant Community Analysis

Canopy Coverage

The overall canopy coverage (Figure 3.2., Table 3.2.) varies among sites primarily because of variations in graminoid and lichen composition. The dominant influences among all sites are soils and previous grazing intensities.

Graminoids represent the largest component of the total vegetation, and the most significant for livestock grazing. Little difference is observable among sites, except for the lower canopy cover on Kluver East and Kluver West. Kluver East apparently is still being influenced by its past grazing intensity, even though it has been excluded from livestock grazing for four growing seasons. Also, a substantial portion (over 50%) of its graminoid composition is contributed by crested wheatgrass. Another third is annual brome grasses. These species are all early season growers, and were present in reduced abundance at the time of sampling. Kluver West supported a high population density of grasshoppers in 1977. This influence was apparent in reduced cover of both graminoids and forbs.

The reason for the striking variation in lichen cover from one site to the next still is obscure. Lichens will be studied more intensively in the coming years.

Mosses never have been a significant component of the vegetation on these sites. Shrubs vary, but this is inherent in the sites, probably because of edaphic and microclimatic differences.

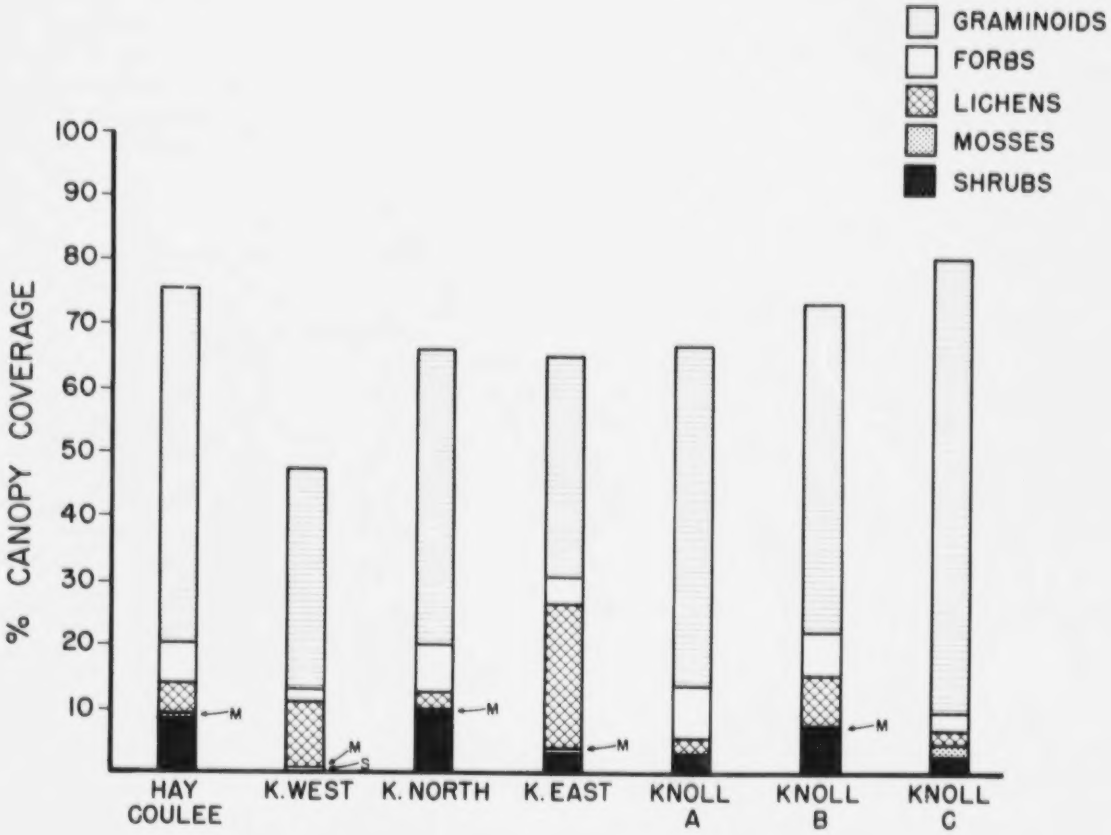


Figure 3.2. Canopy coverage on Colstrip Sites, 1977.

TABLE 3 . 2. CANOPY COVERAGE (PERCENTAGE) FOR COLSTRIP STUDY SITES, 1977.

SPECIES	Hay Coulee 31 Aug.	Kluver West 1 Sept.	Kluver North 1 Sept.	Kluver East 2 Sept.	McRae Knoll A 3 Sept.	McRae Knoll B 3 Sept.	McRae Knoll C 31 Aug.
GRAMINOIDS							
Agropyron cristatum				19.20			
A. smithii	10.95	4.20	3.35	2.65	1.25	2.10	1.65
A. spicatum						7.55	
Aristida longiseta			.05			.05	
Bouteloua gracilis	23.55	2.50	20.50	.65	1.05	8.15	2.20
Bromus japonicus	5.35	1.15	.65	10.40	14.30	.95	18.40
B. tectorum	.05	.15	.15	.15	.90		
Carex eleocharis						.05	
C. filifolia		.95			14.75	5.75	26.80
Koeleria cristata	9.45	.15	.30		.35	13.80	.50
Poa sandbergii	4.65	2.30	2.30	1.00	.60	.15	.25
Schedonnardus paniculatus				.10			
Stipa comata	.60	22.90	18.40	.45	19.75	13.80	21.05
S. viridula	.30						
Vulpia octoflora		.05					
FORBS							
Alyssum desertorum	.10			.05			
Antennaria rosea		.05					
Arabis holboellii					.05		
Aster sp.			.05				
A. falcatus							.05
Astragalus purshii	.05						
Comandra umbellata						.05	
Erysimum asperum	.05			.15	.05	.05	1.00
Gaura coccinea			.05	.35		.05	.40
Grindelia squarrosa						.10	

TABLE 3.2. (continued)

SPECIES	Hay Coulee 31 Aug.	Kluver West 1 Sept.	Kluver North 1 Sept.	Kluver East 2 Sept.	McRae Knoll A 3 Sept.	McRae Knoll B 3 Sept.	McRae Knoll C 31 Aug.
FORBES (continued)							
Lactuca serriola					3.75		
Lomatium sp.	.05						.80
Lugodesmia juncea		.10			2.05		.05
Mammillaria missouriensis					.05		
Medicago sativa						1.50	
Melilotus officinalis						.75	
Opuntia fragilis		.20		.05			
O. polyacantha			.75		1.15	.05	.40
Phlox hoodii	.05			.30		.80	
Polygala alba						1.60	
Polygonum viviparum			.05				
Psoralea argophylla						.10	
Sphaeralcea coccinea	.15				.10	.55	
Taraxacum officinale	2.00	.65	.50	.85	.45		.05
Tragopogon dubius	3.85	1.00	6.05	2.30	.35	.95	.10
HALF-SHRUBS AND SHRUBS							
Artemisia cana	.80				2.85	1.45	
A. dracunculus			.05				.05
A. frigida	2.80	.05	9.50	3.15		2.80	2.30
A. tridentata	4.20						
Atriplex nuttallii						.75	
Ceratoides lanata	.75					.95	
Chrysothamnus nauseosus						.30	
Xanthocephalum sarothrae						.30	

TABLE 3.2. (continued)

SPECIES	Hay Coulee 31 Aug.	Kluver West 1 Sept.	Kluver North 1 Sept.	Kluver East 2 Sept.	McRae Knoll A 3 Sept.	McRae Knoll B 3 Sept.	McRae Knoll C 31 Aug.
OTHERS							
Bare ground	13.25	14.80	22.30	15.15	7.30	23.20	12.85
Litter	73.15	64.75	64.35	58.10	81.55	58.80	74.15
Moss	.80	.10	.05	.05		.45	2.05
Lichens	4.60	11.05	3.20	23.25	2.70	8.40	2.25
Rock	.35	1.05	.10	.10	.40	2.40	1.00
TOTAL VEGETATION	75.55	47.75	65.95	64.90	66.60	73.20	80.30
TOTAL GRAMINOIDS	54.90	34.35	45.70	34.40	52.95	51.30	70.85
TOTAL FORBS	6.70	2.00	7.45	4.05	8.10	6.55	2.80
TOTAL SHRUBS	8.55	.05	9.55	3.15	2.85	6.50	2.35

Species Diversity

Inter-site diversity, evenness, and species richness based on canopy coverage and number are shown in Figure 3.3.

As in the past years, the diversity (cover) at Hay Coulee and Knoll B is significantly higher than that at the other sites. This tendency does not appear at Hay Coulee when diversity is based on plant numbers. Furthermore, number-based diversity is much more variable over all than that derived from cover. This is attributable to variation in annual grass composition. Where large numbers of annual grasses are present, evenness is depressed, resulting in a corresponding reduction in the diversity index.

Species richness varies among sites, with Hay Coulee and Knoll B having the most species. In some instances, notably Kluver North, the low richness has a dampening effect on diversity, although evenness is high.

The sensitivity of diversity indices to differences in sites and range condition has been discussed previously (Taylor *et al.*, 1975). When evenness and richness are examined individually they follow similar patterns and help to elucidate diversity trends.

Plant Phenology

Phenological data are presented in Appendices 3.2 and 3.3.

The most intense phenological sampling in 1977 was done on the McRae Knoll site. We felt that since the Knolls had not received any livestock use for a number of years, there would be no confounding deferment effect which all of the other sites have exhibited since they were fenced in 1974. Further, the Knolls represent a variety of soils, exposures, and plant communities in a relatively small area. Finally, we had more aerial photography and better background data due to a related graduate study on the site.

We have been disappointed in the inability of phenology measurements to reflect subtle seasonal or annual changes in plant community structure. It appears that an adequate phenological baseline would require a number of years to develop, since this parameter is so variable, even in the absence of substantial stress.

Phenology data have been helpful in explaining some species signatures in aerial photography. A key of plant species identification based on phenological changes in photographic signatures will be completed during Spring, 1978. This key should be very useful in interpreting aerial photography on a variety of sites in the Northern Great Plains.

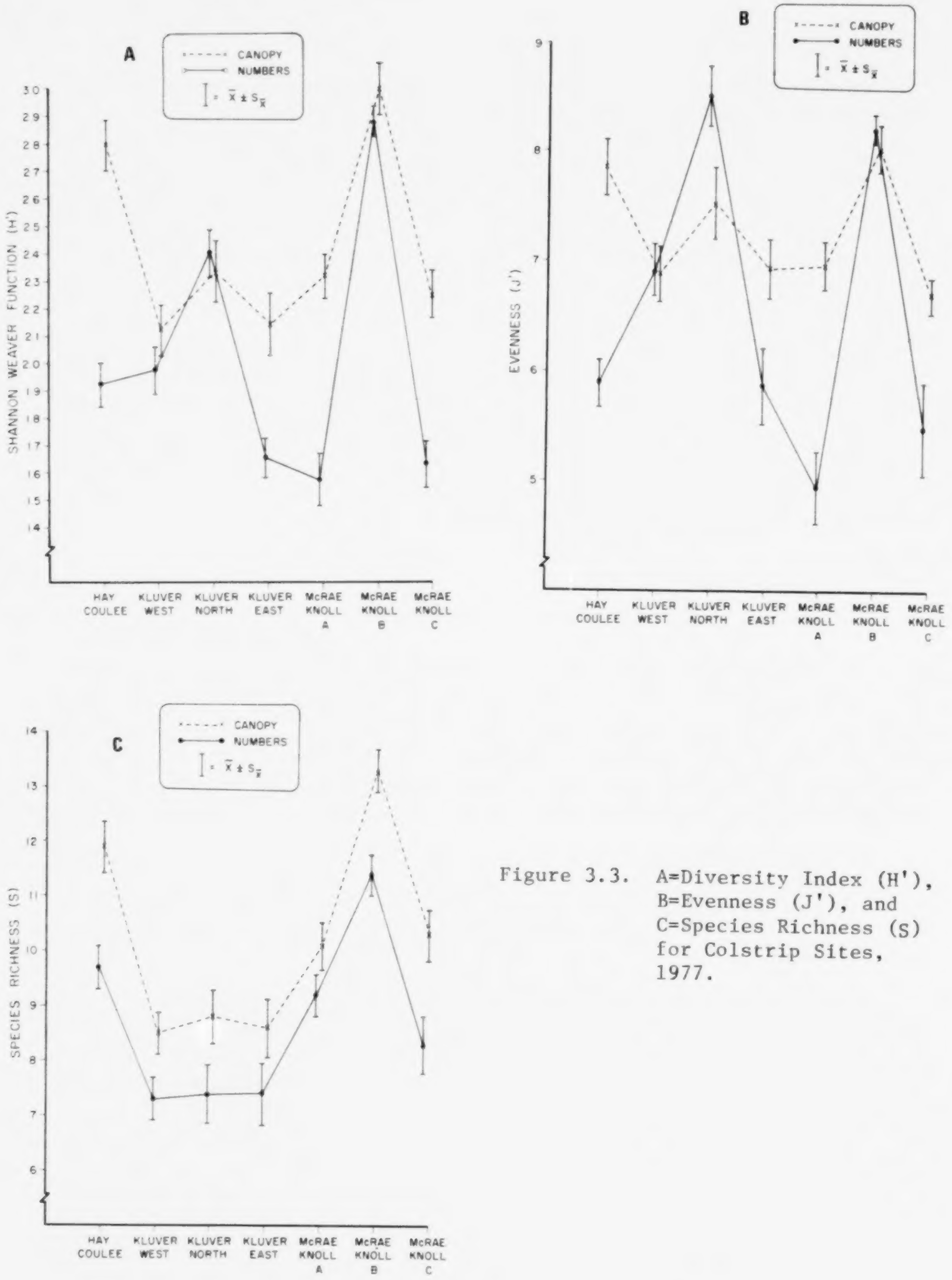


Figure 3.3. A=Diversity Index (H'), B=Evenness (J'), and C=Species Richness (S) for Colstrip Sites, 1977.

Photographic Studies

Ground Level Photography

We are continuing to obtain ground level plot photos and oblique aspect pictures of each study location. This visual record has proven extremely useful in corroborating temporal differences detected in quantitative sampling.

Preliminary photo analysis has suggested that some kinds of quantitative data (cover and density) can be derived with stereoscopic analysis of photo plots. This work will be expanded in the future.

Aerial Photography

Because of the lack of overt stress signs attributable to air pollution, the aerial monitoring of the Colstrip sites is limited to one or two observations annually. The primary value of this photography now is as a continuing record of any subtle changes which might be occurring. In the event of cumulative stress sufficient to trigger undesirable vegetational changes, the rate and nature of such changes will be documented. A list of the aerial photo coverage of the Colstrip sites is included in the comprehensive list in Appendix 24.1.

CONCLUSIONS

A record of plant community diversity, cover, and phenology is continually being collected and refined in the vicinity of Colstrip, Montana. The native vegetational communities represented at the various experimental sites are being thoroughly characterized, so that any changes which may occur due to chronic exposure to air pollutants may be documented.

Thus far, no effects positively linked with air pollution have been observed. It is quite likely that at the level of stack emissions currently being received, it will take a number of years before any effects are noted.

Procedures developed here should have wide application in other grassland situations where coal-fired power plants are being contemplated or operated.

REFERENCES

Daubenmire, R. F. 1959. A Canopy-Coverage Method of Vegetational Analysis. Northw. Sci., 33(1):43-64.

Pielou, E. C. 1969. An Introduction to Mathematical Ecology. J. Wiley & Sons, N.Y. 286 pp.

Shannon, C., and W. Weaver. 1949. Mathematical Theory of Communication. Univ. Illinois Press, Urbana. 117 pp.

Taylor, John E. 1976. Photographic Monitoring of Air Pollution Impacts on Rangeland. Abstr. Ann. Mtg. Soc. Range Mange., Omaha, Nebraska, p. 19.

Taylor, J. E. and W. C. Leininger. 1977. Baseline Vegetational Studies near Colstrip, Montana. Montana State University Mimeo, Bozeman. 67 pp.

Taylor, J. E., W. C. Leininger, and R. J. Fuchs. 1975. Baseline Vegetational Studies near Colstrip. In: Proc. Ft. Union Coal Field Symp., Mont. Acad. Sci., Billings, Montana. pp. 537-551.

Taylor, J. E., W. C. Leininger, and R. J. Fuchs. 1976. Monitoring Plant Community Changes due to Emission from Fossil Fuel Power Plants in Eastern Montana. In: The Bioenvironmental Impact of a Coal-fired Power Plant, Second Interim Report, Colstrip, Montana, June, 1975. R. A. Lewis, N. R. Glass, and A. S. LeFohn, eds. EPA-600/3-76-013, U. S. Environmental Protection Agency, Corvallis, Oregon. pp. 14-40.

Woodcock, W. E. 1976. Aerial Reconnaissance and Photogrammetry with Small Cameras. Photogrammetric Eng. and Remote Sensing, 42(4):503-511.

APPENDIX 3.1.

PLANT SPECIES ENCOUNTERED IN COLSTRIP STUDY SITES, 1974 - 1977.

SYMBOL	SPECIES	Hay Coulee	Kluver East	Kluver West	Kluver North	McRae Knoll A	McRae Knoll B	McRae Knoll C
	GRAMINOIDS							
AGCR	*Agropyron cristatum*		X				X	
AGSM	*A. smithii*	X	X	X	X	X	X	X
AGSP	*A. spicatum*	X				X	X	X
ARLO	*Aristida longiseta*	X	X	X	X	X	X	X
BOCU	*Bouteloua curtipendula*							X
BOGR	*B. gracilis*	X	X	X	X	X	X	X
BRJA	*Bromus japonicus*	X	X	X	X	X	X	X
BRTE	*B. tectorum*	X	X	X	X	X	X	X
CAMO	*Calamagrostis montanensis*				X	X	X	
CALO	*Calamovilfa longifolia*	X	X			X	X	X
CAREX	*Carex* species				X	X	X	X
CAEL	*C. eleocharis*						X	
CAFI	*C. filifolia*	X	X	X	X	X	X	X
KOCR	*Koeleria cristata*	X	X	X	X	X	X	X
MUCU	*Muhlenbergia cuspidata*						X	
ORHY	*Oryzopsis hymenoides*						X	
POA	*Poa* species					X		
POPR	*P. pratensis*	X	X			X	X	
POSA	*P. sandbergii*	X	X	X	X	X	X	X
SCPA	*Schedonnardus paniculatus*	X	X					
SCSC	*Schizachyrium scoparium*			X	X		X	X
SPCR	*Sporobolus cryptandrus*		X		X	X		
STCO	*Stipa comata*	X	X	X	X	X	X	X
STVI	*S. viridula*	X	X				X	
VUOC	*Vulpia octoflora*	X	X	X	X	X	X	X

APPENDIX 3.1. (continued)

SYMBOL	SPECIES	Hay Coulee	Kluver East	Kluver West	Kluver North	McRae Knoll A	McRae Knoll B	McRae Knoll C
	FORBS							
ACMI	*Achillea millefolium*	X	X	X	X		X	
AGGL	*Agoseris glauca*			X				
ALTE	*Allium textile*	X			X	X	X	X
ALDE	*Alyssum desertorum*	X	X					
AMPS	*Ambrosia psilostachya*					X		X
ANOC	*Androsace occidentalis*	X	X	X	X	X	X	X
ANPA	*Anemone patens*	X						
ANTEN	*Antennaria* species	X	X	X	X	X		X
ANRO	*A. rosea*			X				
ARHO	*Arabis holboellii*	X			X	X	X	X
ARLU	*Artemisia ludoviciana*	X						
ASTER	*Aster* species	X		X	X		X	X
ASCA	*A. campestris*						X	
ASFA	*A. falcatus*							X
ASTRA	*Astragalus* species			X			X	
ASCR	*A. crassicarpus*	X		X	X	X		
ASGI	*A. gilviflorus*	X						
ASPU	*A. purshii*	X						
ASST	*A. striatus*	X						
CANU	*Calochortus nuttallii*	X	X	X	X	X	X	X
CASE	*Castilleja sessiliflora*				X		X	
CEAR	*Cerastium arvense*					X	X	
CIUN	*Cirsium undulatum*		X	X	X			X
COLI	*Collomia linearis*	X	X	X			X	
COUM	*Comandra umbellata*	X					X	
COCA	*Conyza canadensis*	X	X	X	X			X
CREPI	*Crepis* species		X		X	X		
CRYPT	*Cryptanthe* species	X						
DEBI	*Delphinium bicolor*	X	X		X			X
DESCU	*Descurainia* species					X		X

APPENDIX 3.1. (continued)

SYMBOL	SPECIES	Hay Coulee	Kluver East	Kluver West	Kluver North	McRae Knoll A	McRae Knoll B	McRae Knoll C
	FORBS (cont.)							
DRABA	*Draba* species	X	X	X		X	X	X
ECPA	*Echinacea pallida*			X			X	X
ERIGE	*Erigeron* species					X	X	X
ERDI	*E. Divergens*		X	X	X			
ERPU	*E. pumilus*	X				X	X	
ERAN	*Eriogonum annuum*			X				X
ERAS	*Erysimum asperum*	X	X	X	X	X	X	X
EVPI	*Evolvulus pilosus*		X	X	X			
GACO	*Gaura coccinea*	X	X	X	X	X	X	X
GRSQ	*Grindelia squarrosa*	X		X	X		X	
HASP	*Haplopappus spinulosus*	X						
HEHI	*Hedeoma hispida*	X	X	X	X	X	X	X
HEVI	*Heterotheca villosa*	X	X					X
HYFI	*Hymenopappus filifolius*	X						
LAPU	*Lactuca pulchella*					X		
LASE	*L. serriola*	X		X	X	X	X	X
LEPID	*Lepidium* species	X	X	X	X	X		X
LEDE	*L. densiflorum*	X		X			X	X
LEMO	*Leucocrinum montanum*		X	X	X	X	X	X
LIPU	*Liatris punctata*		X					X
LIPE	*Linum perenne*	X	X			X	X	
LIRI	*L. rigidum*	X		X	X	X	X	
LIIN	*Lithospermum incisum*							X
LIRU	*L. ruderale*					X		
LOMAT	*Lomatium* species	X				X		
LOOR	*L. orientale*	X	X	X			X	
LYJU	*Lygodesmia juncea*		X	X	X	X	X	X
MAMI	*Mammillaria missouriensis*		X	X	X	X	X	X
MESA	*Medicago sativa*					X	X	X
MEOF	*Melilotus officinalis*	X	X		X	X	X	X

APPENDIX 3.1. (continued)

SYMBOL	SPECIES	Hay Coulee	Kluver East	Kluver West	Kluver North	McRae Knoll A	McRae Knoll B	McRae Knoll C
	FORBS (cont.)							
MILI	*Mirabilis linearis*				X			
OESE	*Oenothera serralata*							X
OPUNT	*Opuntia* species			X				
OPFR	*O. fragilis*	X	X	X	X	X		X
OPPO	*O. polyacantha*	X	X	X	X	X	X	X
ORLU	*Orthocarpus luteus*				X			
OXYTR	*Oxytropis* species	X					X	X
PENST	*Penstemon* species	X	X	X	X			
PEAL	*P. albidus*	X	X	X			X	
PEPU	*Petalostemon purpureum*		X				X	
PHLOX	*Phlox* species	X		X			X	
PHHO	*P. hoodii*	X	X	X	X		X	
PLPA	*Plantago patagonica*	X	X	X	X	X	X	
PLSP	*P. spinulosa*	X	X					
POAL	*Polygala alba*	X	X				X	X
POVI	*Polygonum viviparum*				X			
PSAR	*Psoralea argophylla*	X	X	X	X	X	X	X
PSES	*P. esculenta*				X			
RACO	*Ratibida columnifera*	X	X	X	X		X	
SENEC	*Senecio* species							X
SECA	*S. canus*	X		X		X	X	
SIAL	*Sisymbrium altissimum*		X		X	X		X
SOLID	*Solidago* species	X			X		X	X
SOMI	*S. missouriensis*	X	X	X		X	X	X
SOOC	*S. occidentalis*				X			
SPCO	*Sphaeralcea coccinea*	X	X	X	X	X	X	X
TAOF	*Taraxacum officinale*	X	X	X	X	X	X	X
TRDU	*Tragopogon dubius*	X	X	X	X	X	X	X
VINU	*Viola nuttallii*	X						
YUGL	*Yucca glauca*					X	X	X

APPENDIX 3.1. (continued)

SYMBOL	SPECIES	Hay Coulee	Kluver East	Kluver West	Kluver North	McRae Knoll A	McRae Knoll B	McRae Knoll C
	FORBS (cont.)							
ZYVE	*Zygadenus venenosus*		X			X	X	
	HALF-SHRUBS AND SHRUBS							
ARCA	*Artemisia cana*	X	X		X	X	X	X
ARDR	*A. dracunculus*	X	X	X	X	X	X	X
ARFR	*A. frigida*	X	X	X	X	X	X	X
ARTR	*A. tridentata*	X	X			X	X	
ATCA	*Atriplex canescens*	X						
ATNU	*A. nuttallii*						X	
CELA	*Ceratoides lanata*	X	X		X		X	
CHNA	*Chrysothamnus nauseosus*						X	
JUSC	*Juniperus scopulorum*						X	
PRVI	*Prunus virginiana*					X		X
RHTR	*Rhus trilobata*					X	X	X
ROSA	*Rosa* species	X						
XASA	*Xanthocephalum sarothrae*	X	X			X	X	X

APPENDIX 3.2.

PHENOLOGICAL PROFILE FOR COLSTRIP VALIDATION SITES, 1977[1]

PHENOLOGICAL CODE

SPECIES	Hay Coulee 3 Sept.	Kluver West 1 Sept.	Kluver North 1 Sept.	Kluver East 2 Sept.
GRAMINOIDS				
Agropyron cristatum				11 & 3
A. smithii	3 & 12	10	11	12
A. spicatum	12			
Aristida longiseta	11	12	11	11
Bouteloua gracilis	12	12	11	11
Bromus japonicus	12	12	12	12
B. tectorum	12	12	12	
Calamovilfa longifolia	11			
Carex filifolia	3	12	12	
Koeleria cristata	3 & 12	12	12	12
Poa sandbergii	3 & 12	12	12	12
Schedonnardus paniculatus				4
Sporobolus cryptandrus		12	11	
Stipa comata	3	12	12	12
S. viridula	3			12
FORBS				
Achillea millefolium	3 & 12			12
Alyssum desertorum	12			12
Ambrosia psilostachya			11	
Antennaria species		12	12	
Asclepias verticillata				3
Aster species			10	
Astragalus drummondii			12	
A. purshii	12			
Calochortus nuttallii			12	12
Chaenactis douglasii	12			
Chenopodium album			11	
Cirsium undulatum		3 & 12	3 & 11	3 & 11
Erigeron divergens	8 & 11			
Eriogonum annuum				8
Erysimum asperum	3 & 12	12	12	3
Evolvulus pilosus		12	12	12
Gaura coccinea	12	12	12	12
Grindelia squarrosa	8	8	3 & 8	8
Heterotheca villosa			12	
Lactuca serriola	11		12	
Linum perenne	12			12

---

[1] Codes are given on p.110.

APPENDIX 3.2. (continued)

PHENOLOGICAL CODE

SPECIES	Hay Coulee 3 Sept.	Kluver West 1 Sept.	Kluver North 1 Sept.	Kluver East 2 Sept.
FORBS (cont.)				
Lomatium species	12			
Lygodesmia juncea		12		9
Mammillaria missouriensis			12	
Melilotus officinalis		12		
Opuntia fragilis	12	12	12	12
O. polyacantha	12		12	12
Penstemon species			12	
Phlox hoodii	12			
Plantago spinulosa	12			
Polygala alba	12			
Polygonum viviparum			11	
Psoralea argophylla		12	12	
Ratibida columnifera			12	12
Senecio canus	12			
Sisymbrium altissimum				12
Solidago missouriensis	9			
Sphaeralcea coccinea	12		12	
Taraxacum officinale	3 & 12	3 & 12	3	3
Tragopogon dubius	3 & 12	12	12	3 & 12
Artemisia cana	9		10	
A. dracunculus	9	6	10	
A. frigida	9	6	10	
A. tridentata	9			
Ceratoides lanata	10		11	
Prunus virginiana	12			
Xanthocephalum sarothrae	8			

APPENDIX 3.3.

PHENOLOGICAL PROFILE FOR McRAE KNOLLS, 1977 [1]

(Pooled for 3 Knolls)

PHENOLOGICAL CODE

SPECIES	April 14	April 27	June 3	June 27	July 22	Sept. 3
GRAMINOIDS						
Agropyron smithii	1	3	3	4		3 & 12
A. spicatum	1	3	6	7	11	12
Aristida longiseta	1	1	4	7	10	11
Bouteloua curtipendula				3	10	11
B. gracilis	1	2	3	3	11	12
Bromus sp.*	2	3				3
B. inermis	2	3	5	8	11	11-12
B. japonicus			6	10	11	12
B. tectorum			6	11	12	12
Calamovilfa longifolia	12	1	3	3	8	9
Carex filifolia	4	8	10	11	12	3
Elymus canadensis					9	
Koeleria cristata	3	4	8	10	11	3
Muhlenbergia cuspidata			3	3	8	9
Oryzopsis hymenoides			6	10	11	12
Poa pratensis	2	3	8	11	12	12
P. sandbergii	3	4	8	11	12	3 & 12
Schizachyrium scoparium	12	12	3	3	7	11
Sporobolus cryptandrus				3		
Stipa comata	1	3	6	9	11	3 & 12
S. viridula	1	3	5	9	12	12
Vulpia octoflora			7			12
FORBS						
Achillea millefolium	1	3	7	9	10	12
Allium textile	3					
Ambrosia psilostachya	12	12	3	3	5	9
Androsace occidentalis	1	5	12	12	12	12
Antennaria sp.		4		12	12	12
Arabis holboellii			10	11	12	12
Artemisia ludoviciana	12	3	3	3	5	9

* Includes B. japonicus and B. tectorum, which were indistinguishable on some dates.

---

[1] Codes are given on p.110.

APPENDIX 3.3. (continued)

PHENOLOGICAL CODE

SPECIES	April 14	April 27	June 3	June 27	July 22	Sept. 3
FORBS (cont.)						
Asclepias sp.						11
Aster campestris					8	
A. falcatus	12	12	4	4	5	9
Astragalus crassicarpus		3	9	10-11		12
A. gilviflorus	2	3			4	12
A. purshii				9		
Calochortus nuttallii			6	9	12	12
Camelina microcarpa			9	9	12	12
Campanula rotundifolia				8		
Castilleja sessiliflora			7			
Chaenactis douglassii			5	9	10	12
Chenopodium album					8	12
Cirsium undulatum	1	3	3	1 & 8	3 & 10	3 & 12
Cleome serrulata						10
Comandra umbellata	2	5	5	9	12	12
Crepis sp.	1	3	9			12
Cryptanthe bradburiana		8				
Delphinium bicolor	3	7				
Echinacea pallida	12	1	4	8	9	12
Erigeron divergens			7	8		
Eriogonum annuum	3			3		8
E. multiceps	2	3	3	4	8	
Erysimum asperum	3	5	9	10		3 & 12
Evolvulus pilosus					4	12
Gaura coccinea			6	7	12	12
Gilia congesta					12	12
Grindelia squarrosa	1	2	3	4	5	8
Helianthus annuus				8	10	11
Heterotheca villosa	1	1	4	6	8	9
Lactuca pulchella		2	3	3	10	12
L. serriola	12	2	3	3	10	12
Leucocrinum montanum	4	4	12	12	12	12
Liatris punctata						9
Linum perenne			7	10	12	12
L. rigidum					10	11
Lithospermum incisum			5	9	10	12
Lomatium orientale	6	8	11	12	12	12
Lygodesmia juncea			5	8	10	10
Medicago sativa	1	3	5	7	10	11
Melilotus officinalis	12					
Musineon divaricatum	6	8		12		

APPENDIX 3.3. (continued)

PHENOLOGICAL CODE

SPECIES	April 14	April 27	June 3	June 27	July 22	Sept. 3
FORBS (cont.)						
Oenothera serrulata			6	9		10
Opuntia fragilis					12	12
O. polyacantha	12	2	5	6	10	12
Oxytropis sp.					12	12
O. besseyi	1	3	7	8	11	12
O. lambertii	1	2	5	10	12	12
Penstemon sp.	1					
P. albidus			7	9	12	12
Petalostemon purpureum			5	7	9	11
Phlox hoodii	6	9			12	12
Plantago patagonica						12
Polygala alba		2	7	8	11	3 & 12
Potentilla sp.	2	2	4	7		
Psoralea argophylla	12		5	8	11	12
P. tenuiflora			5	8	10	12
Ratibida columnifera		2	5	7	10	11
Smilacina sp.						10
Solidago missouriensis	1	3	3	4	7	9
S. mollis						9
S. occidentalis	1	2	3	3	6	8
Sphaeralcea coccinea			6	8	12	12
Taraxacum officinale	2	7	12	12	12	3 & 12
Tradescantia occidentalis		3		6	12	12
Tragopogon dubius	2	3	7	12	3 & 12	3 & 12
Vicea americana						12
Viola nuttallii		5		10	12	
Yucca glauca	1	3	5	10	11	12
Zygadenus venenosus	2	4	9	10	12	12
HALF-SHRUBS AND SHRUBS						
Artemisia cana	1	3	3	3	5	9
A. dracunculus	1	3	3	4	6	9
A. frigida	1	3	3	3	5	9
A. tridentata	1	3	3	3	5	
Atriplex nuttallii			4		9	10
Ceratoides lanata	1	3	7	8	9	10
Chrysothamnus nauseosus	1	3	3	3	6	8
Rhus trilobata	1	5	9	10	10	11
Ribes aurem	1	6	9	9	10	12
R. setosum					12	12

APPENDIX 3.3. (continued)

PHENOLOGICAL CODE

SPECIES	April 14	April 27	June 3	June 27	July 22	Sept. 3
HALF-SHRUBS AND SHRUBS (cont.)						
Rosa arkansana			4			
R. woodsii	1	2	7	9	10	10
Symphoricarpos occidentalis	12	1	5	8	9	10
Xanthocephalum sarothrae	1	2	3	3	5	8
TREES						
Acer negundo	12	2	8	12	12	12
Amelanchier alnifolia		7		9	12	12
Fraxinus pennsylvanica	12	2	3	3	12	12
Juniperus scopulorum				9	12	12
Populus deltoides				12	12	12
Prunus virginiana	1	3	9	9	10	11
Shepherdia argentea	1	2	3	3	10	12

SECTION 4

SUMMARY OF OBSERVATIONS OF *USNEA HIRTA* AND *PARMELIA CHLOROCHROA* IN THE COLSTRIP AREA, 1974-77

S. Eversman

ABSTRACT

In this portion of the lichen project, I have been monitoring the respiration rates, total sulfur content, percentage of algal plasmolysis, and general thallus appearance of two lichen species since 1974. The respiration rates of *Usnea hirta* (L.) Wigg. and *Parmelia chlorochroa* Tuck. samples rose significantly in September, 1977, compared to previously collected samples. *Usnea hirta* demonstrated a small decrease in relative absorbance of light by chlorophyll from 1975 to 1977.

INTRODUCTION

The objectives of this portion of the lichen project have been to: 1) identify baseline lichen community information for the grassland and ponderosa pine vegetation types; 2) establish baseline anatomical and physiological conditions of two native lichen species, *Usnea hirta* (L.) Wigg. and *Parmelia chlorochroa* Tuck., and 3) continually monitor the species and communities to detect changes in them that may be attributable to coal-burning power plants.

The major epiphytic lichen on the ponderosa pine tree is *Usnea hirta*. A major terricolous lichen is *Parmelia chlorochroa*. I have collected and stored samples of these lichens since 1975, recording respiration rates, percentages of plasmolyzed algal cells and general thallus condition, and determining sulfur contents. In 1977, chlorophyll absorption was also measured.

In addition to analysis of individual samples, I have been annually recording terrestrial community composition using a point-drop method (Eversman, 1978) in designated exclosure in the Colstrip area. Epiphytic lichen communities on the ponderosa pine sites in the Colstrip area and in parts of the Custer National Forest have been determined by recording percentage of epiphytic lichen coverage on tree trunks by species.

Traditionally, epiphytic community analyses in polluted areas have indicated disappearance of lichens (and mosses) in areas close to a polluted source, with progressive reappearance of species as distance from the source increases. The ponderosa pines in the immediate Colstrip area, i.e., within 1-20 km, support a very sparse epiphytic community compared with areas of the Custer National Forest to the south and east. This was true before the power generating plants were constructed. Therefore, a completely community-oriented approach has been impractical. Emphasis has been, and will continue to be, on the characteristics of individual lichen species, with comparisons between years and with results from the SO_2 fumigation studies (ZAPS sites).

MATERIALS AND METHODS

The sites and methods have been described previously (Eversman, 1977). Three ponderosa pine sites, P17, P18, and P19, were added in 1977; site P9 was deleted. *Usnea hirta* samples from site P10 were transplanted to the new sites in April, 1977, and samples were collected in September, 1977. I collected *Parmelia chlorochroa* from the four primary grassland sites and *Usnea hirta* from the ponderosa pine sites in July and September each year (Figure 4.1).

Respiration rates were determined manometrically for all samples collected 1975-77. Chlorophyll extract absorbance was determined for 300-mg samples by making three 3-ml methanol extractions, adding methanol to 10 ml, then reading absorbance on a Beckman DU spectrophotometer at 665 nm. Algal cell counts are made by making 3 microscope slides of lichen thallus, and counting 100 cells on each slide recording the numbers of plasmolyzed algal cells.

RESULTS AND DISCUSSION

This report includes only results of individual species analysis from 1977 that can be compared with 1974-76 information and with the ZAPS sites results.

Table 4.1 gives annual readings of respiration rates of *Parmelia chlorochroa* samples by grassland site. Analysis of variance of most sites indicated that the samples collected September 1977 had a significantly ($P < .01$) greater respiration rate than samples collected at any other time. A comparison of all samples grouped by month and year indicated that respiration rates of *P. chlorochroa* samples collected from the ground on the ZAPS sites and from the grassland sites showed no significant differences among ZAPS and field sites in 1975 and 1976.

Comparisons of relative chlorophyll absorbance at 665 nm (Table 4.2) of field samples and those from ZAPS sites (10-cm height, ZAPS I site, September, 1977, Table 17.7) indicated that the field sample chlorophyll content, except for Kluver East and Hay Coulee samples, was generally higher than chlorophyll content of those of ZAPS samples. There was no significant difference among field (Colstrip area grassland) sites.

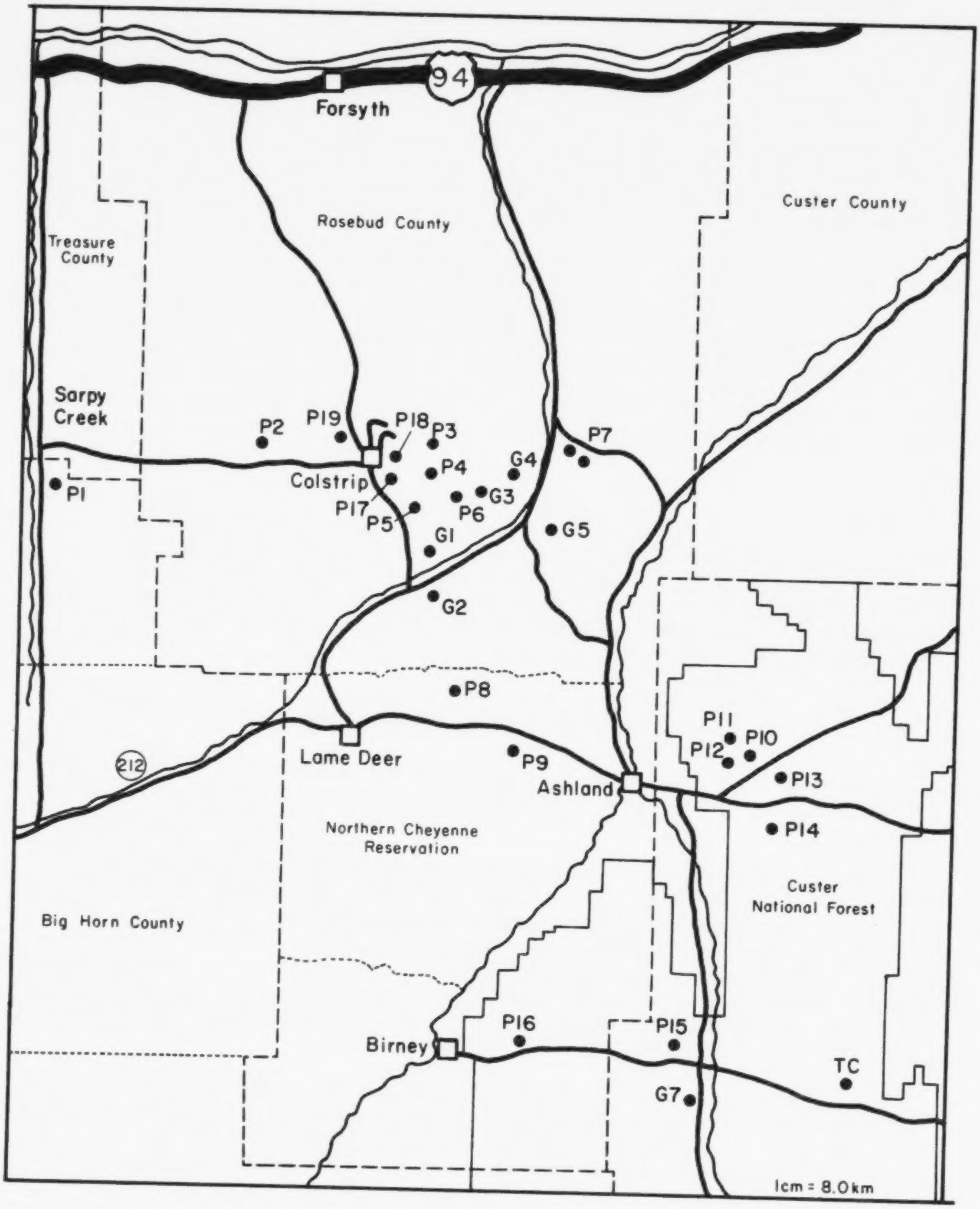

94
Forsyth
Rosebud County
Custer County
Treasure County
Sarpy Creek
P1
P2
P19
P18
P3
P4
Colstrip
P17
P5
P6
G1
G3
G4
P7
G5
G2
P8
212
Lame Deer
P9
Ashland
P11
P10
P12
P13
P14
Northern Cheyenne Reservation
Custer National Forest
Big Horn County
P16
P15
Birney
TC
G7
1cm = 8.0km

Figure 4.1. Locations of lichen study sites in the Colstrip area. P1-19 are ponderosa pine sites; G1-7 are grassland sites. TC is ZAPS I site.

TABLE 4.1. RESPIRATION RATES OF *PARMELIA CHLOROCHROA* SAMPLES COLLECTED FROM GRASSLAND SITES 1974-1977.

Location	Collection Number	Dates	$\bar{X}^*$	$CI_{.95}$	ANOVA†	F
G1. Hay Coulee	1	7-20-74	314	35	$8\ 4\ \underline{2\ 1\ 7}\ 5\ 6\ 3$ $\underline{}$	83.87
	2	8-30-74	332	68		
	3	6-26-75	215	31		
	4	5-14-76	458	53		
	5	7-16-76	290	47		
	6	9-15-76	274	55		
	7	7-07-77	310	72		
	8	9-13-77	585	85		
G2. McRae Knolls	1	9-15-76	341	52	$\underline{2\ 1}$	0.070
	2	7-07-77	367	420		
G3. Kluver West	1	7-21-74	218	25	$\underline{7\ 5}\ \underline{4\ 2\ 3\ 6\ 1}$	21.15
	2	7-07-75	293	36		
	3	7-16-76	272	26		
	4	9-15-76	302	37		
	5	6-20-77	505	408		
	6	7-17-77	252	67		
	7	9-13-77	561	586		
G4. Kluver North	1	7-21-74	342	29	$6\ \underline{2\ 1}\ 5\ \underline{4\ 3}$	112.49
	2	7-07-75	349	26		
	3	7-16-76	242	9		
	4	9-15-76	262	85		
	5	7-07-77	314	104		
	6	9-13-77	556	127		
G5. Kluver East	1	7-21-74	227	54	$6\ \underline{3\ 4\ 2\ 5}\ 1$	35.55
	2	6-26-75	317	50		
	3	7-14-76	322	65		
	4	9-15-76	321	98		
	5	7-07-77	301	209		
	6	9-13-77	512	75		
Other						
Kluver NE-1		9-13-77	606	129		
BNW #2		9-13-77	420	127		
Kluver E-1		9-13-77	546	455		
Pasture		5-14-76	371	86		

---

*Respiration rates are expressed as mean μl O_2 consumed $g\text{-}hr^{-1}$, of 3 samples with ± .95 confidence interval.

†Results of one-way analysis of variance (ANOVA) and the F value. Samples not sharing the same underline are significantly different ($P < .05$).

TABLE 4.2. RELATIVE ABSORBANCE AT 665 nm LIGHT BY CHLOROPHYLL EXTRACTS OF *PARMELIA CHLOROCHROA* AND *USNEA HIRTA* SAMPLES COLLECTED 1975-1977 FROM GRASSLAND AND PONDEROSA PINE SITES.

Usnea hirta				*Parmelia chlorochroa*			
Collection Site	Date	$\bar{X}$*	CI $_{.95}$	Collection Site	Date	$\bar{X}$	CI $_{.95}$
East Otter Creek	9-15-75	.273	.219	Kluver West	6-25-75	.154	.228
	9-15-75	.258	.051		6-23-77	.204	.043
	3-25-77	.211	.086		9-13-77	.162	.065
SEAM 1	7-16-75	.294	.086	Kluver North	6-25-75	.075	.034
	7-06-77	.230	.125		9-13-77	.176	.043
	7-06-77	.246	.116				
SEAM 2	7-16-75	.373	.030	Kluver East	6-25-76	.081	.052
	7-06-77	.221	.034		9-14-77	.112	.034
Home Creek Butte	7-16-75	.251	.095	Hay Coulee	5-14-76	.103	.034
	9-15-77	.240	.010		7-07-77	.108	.060
					9-14-77	.149	.060
Ft. Howes - tr	9-15-76	.223	.146	ZAPS I A	9-14-77	.039	.034
- tr	8-17-77	.243	.138	B	9-14-77	.116	.006
nat	8-17-77	.188	.095	C	9-14-77	.066	.051
Poker Jim - tr	9-15-76	.168	.069	D	9-14-77	.031	0
nat	7-06-77	.156	.056				
tr	7-06-77	.120	.077	Other Sites			
				Kluver NE-1	9-13-77	.232	.102
MVS - nat	9-17-75	.213	.013	Kluver E-1	9-13-77	.124	.069
nat	7-07-77	.378	.142				
tr	7-07-77	.174	.047				

* Values are given as mean relative absorbance of 3 samples with ± .95 confidence interval

Table 4.3 gives respiration rates for *Usnea hirta* samples from 18 ponderosa pine sites, with analysis of variance for most sites between years of collection. Site P10 (East Otter Creek) and P15 (Fort Howes Ranger Station) are considered to be the control sites. The ground-slope of P10 faces southeast, away from the power plant; Fort Howes is a north-facing slope separated by many hills from the power plants. Other sites are ridges facing Colstrip.

The latest collection at each site, generally September 1977, has the highest rate of respiration for most of the sites. These sites, including grassland sites, are clustered in the Colstrip area, and extend to the southeast for 70 km. Analyses of variance give these results: 1) Field samples from all sites in July and September 1975, 1976, and 1977, have significantly higher respiration rates than samples from ZAPS D (I and II). 2) In 1975 and 1976, there are significant differences between field sites and samples from ZAPS C, I and II. The results are less clear-cut in 1977. Part of this is because of the indistinct differences obtained on the ZAPS sites, plots B and C (Table 17.6). 3) There are no regular or significant differences between samples from ponderosa pine sites and those from ZAPS A and B. Samples from the immediate Colstrip areas, P17, P18, and P19, exhibited the same sharp increase in respiration rate obtained on ZAPS I-B and II-A sites. This is interpreted as a stress response, but whether or not it is due to transplanting stress, of SO_2 or both, is not yet certain.

Results of chlorophyll absorption determinations of *U. hirta* in 1975-1977 are in Table 4.2. There seems to be a general area-wide decrease, but not significant, in chlorophyll absorption from 1975-1977. The total mean values decreased from 0.277 in 1975, to 0.211 in 1976, to 0.207 in 1977.

CONCLUSIONS

There may be indications of stress on lichens in the Colstrip area. There are sharp respiration rate increases in September 1977 in both *Usnea hirta* and *Parmelia chlorochroa* samples collected at sites closest to Colstrip, and a general decrease in chlorophyll absorption in *U. hirta*.

TABLE 4.3 RESPIRATION RATES OF *USNEA HIRTA* SAMPLES COLLECTED FROM PONDEROSA PINE SITES 1975-1977.

Location Ω	Collection Number	Date	$\bar{X}^*$	$CI_{.95}$	ANOVA †	F
P1. Sarpy Creek ‡	1	9-15-75	640	30	3 2 1	0.93
	2	9-16-76	658	323		
	3	9-12-77	694	380		
P2. Castle Rock ‡	1	9-15-75	640	30	1 3 2	8.71
	2	9-14-76	499	214		
	3	9-12-77	588	124		
P3. Kluver NE-1 ‡	1	7-08-77	620	221	1 2	0.50
	2	9-13-77	660	57		
P4. Kluver E-1 ‡	1	9-15-75	640	30	3 4 1 2	2.49
	2	9-15-76	573	133		
	3	7-07-77	645	77		
	4	9-13-77	642	62		
P5. McRae Hill ‡	1	9-13-75	640	30	2 1	8.04
	2	9-13-77	750	271		
P6. Kluver West trees ‡	1	7-16-78	669	124	1 2 3 4	6.28
	2	9-15-76	633	132		
	3	9-13-77	600	5		
	4	6-20-77	519	127		
P7. Diamond Ranch Buttes ‡	1	6-23-76	744	112	1 2 3	3.76
	2	6-23-77	629	80		
	3	6-23-77	578	298		
P8. Morning Star View ‡	1	9-15-75	640	30	2 3 1	4.03
	2	9-19-76	828	153		
	3	7-07-77	643	134		
native	1	7-07-77	728	75		
P10. East Otter Creek (transplant source)	1	3-23-76	709	84	2 1 4 5 3	12.57
	2	6-23-76	744	112		
	3	7-15-76	463	142		
	4	8-09-76	668	102		
	5	9-15-76	633	132		
	1	4-27-77	743	159	1 4 2 6 3 5	3.85
	2	6-21-77	695	112		
	3	7-07-77	620	100		
	4	8-04-77	735	99		
	5	8-16-77	516	27		
	6	9-15-77	681	136		

TABLE 4.3 (Cont.)

Location		Collection Number	Date	$\bar{X}^*$	$CI_{.95}$	ANOVA	F
P11.	Seam	1	7-16-75	545	25	3 1 2	4.42
	Site 1	2	7-15-76	454	27		
		3	7-06-77	746	94		
P12.	Seam	1	7-16-75	548	18	3 1 2	14.59
	Site 2	2	7-15-76	497	119		
		3	7-06-77	629	62		
P13.	Home	1	7-16-75	462	38	3 2 1	28.17
	Creek	2	7-15-76	486	119		
	Butte	3	9-15-77	765	244		
P14.	Three	1	7-16-75	528	25	3 2 1	22.55
	Mile	2	8-09-76	667	189		
	top	3	9-15-76	692	80		
	bottom	1	7-16-75	539	43	2 1 3	0.51
		2	8-09-76	547	99		
		3	9-15-76	500	215		
P15.	Fort	1	5-13-75	829	42		
	Howes	2	9-15-76	597	107	1 4 3 2	18.63
	transplants	3	6-21-77	675	52		
		4	8-17-77	688	119		
	natives	1	5-13-75	493	54	2 4 1 5 3	1.13
		2	5-14-76	576	281		
		3	9-15-76	479	124		
		4	6-21-77	543	99		
		5	8-17-77	487	119		
P16.	Poker Jim	1	9-15-75	640	30	2 1 3	0.90
	Butte	2	9-15-76	669	185		
	transplants	3	7-06-77	615	144		
	natives	1	7-17-75	630	52	2 4 1 3 5	12.03
		2	5-13-76	794	456		
		3	7-16-76	610	77		
		4	9-15-76	654	129		
		5	7-06-77	452	47		
P17.	BNW	1	4-27-77	743	159	2 1	3.84
	#1	2	9-13-77	964	395		

TABLE 4.3. (Cont.)

Location	Collection Number	Date	$\bar{X}^*$	$CI_{.95}$	ANOVA[†]	F
P18. BNW # 2	1	4-27-77	743	159	2 3 1	9.06
	2	9-13-77	1006	55		
F19. BNW # 3	1	4-27-77	743	159	2 1	4.21
	2	9-13-77	935	387		

[Ω] Location P9 abandoned after serving as source for 1975 transplants.

[*] Respiration rates are expressed as μl O_2 consumed $g\text{-}hr^{-1}$, the mean of 3 samples with ± .95 confidence interval (CI).

[†] Results of one-way analysis of variance (ANOVA) and the F value. Samples not sharing the same underline are significantly different ($P < .05$).

[‡] Transplants from East Otter Creek site.

REFERENCE

Eversman, S. 1978. Soil and Epiphytic Lichen Communities of the Colstrip, Montana Area. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Third Interim Report, Colstrip, Montana. E. M. Preston and R. A. Lewis, eds. EPA-600/3-78-021, U. S. Environmental Protection Agency, Corvallis, Oregon. pp. 50-64.

SECTION 5

FOLIAR PATHOLOGIES OF PONDEROSA PINE NEAR COLSTRIP

C. C. Gordon, P. C. Tourangeau, P. M. Rice

ABSTRACT

The growth/health/damage characteristics of four different years of pine foliage collected during 1977 at "pristine" and chronically-polluted sites are compared and discussed. Growth characteristics of needle cross-sectional areas and needle lengths are compared within and between sites via anova. Fluoride and sulfur concentrations found in needles and fascicular sheaths from 1977 pine foliage samples are compared with the findings of 1975 and 1976 studies reported in the EPA Third Interim Report (TIR). Similar data obtained from four ponderosa pine-skunkbush sites being studied with DOE funding in southeastern Montana are also reported.

Data are presented on the growth/health/damage characteristics, such as needle mottling, tip necrosis, and insect damage on pine foliage exposed for different time periods in chronically-polluted areas. This portion of our study demonstrates that all air pollution damage symptoms manifested by the pine foliage being studied can be and are mimicked macroscopically by abiotic and biotic causal agents in pristine environments. The foliar symptoms being measured over time are shown to increase or decrease in comparison to those found in pristine areas.

A discussion is presented on a tentative conceptual model of chronic air pollution damage to conifer species. This model is based on sulfur and fluoride levels in different-aged foliage, as well as on the increases and decreases of average amounts of growth/health/damage characteristics of pine foliage over extended exposure periods.

Fluoride levels found in the foliage of understory species from the ponderosa pine-skunkbush sites are also reported.

INTRODUCTION

This portion of our EPA-CERL-sponsored studies (Corvallis, Oregon, Environmental Research Laboratory) in southeastern Montana discusses the implications of the growth/health/damage characteristics of ponderosa pine foliage collected from permanent sites located at various distances from Colstrip, Montana, the site of two 350 MW power plants. Our study also includes the determination of sulfur and fluoride levels in this foliage and selected understory species of shrubs, forbs, and grasses. Quantification of the growth/health/damage characteristics of ponderosa pine foliage collected from other polluted and pristine ecosystems have rarely been reported in the scientific literature before studies were initiated in southeastern Montana; in fact, according to the literature, these characteristics were only occasionally measured and quantified for any coniferous species.

It was our original hypothesis that if the growth/health/damage characteristics of ponderosa pine foliage from southeastern Montana could be established and quantified before the two Colstrip 350 MW units began operating in late 1975 and mid-1976, any impacts from the chronic levels of the power plants' gaseous and particulate emissions upon the foliage could be ascertained in the future. Ponderosa pine is the dominant tree species in southeastern Montana and is the only one utilized for timber production throughout the area. Its susceptibility to phytotoxic gases such as SO_2, O_3, HF, and/or combinations of these gases has been well established by the field studies of Katz and McCallum (1939), Scheffer and Hedgcock (1955), Compton *et al.* (1960, 1968), Carlson (1974), Carlson and Dewey (1971), Evans and Miller (1972), Treshow *et al.* (1967), Cobb *et al.* (1968), Gordon (1974), Miller *et al.* (1977), and Tourangeau *et al.* (1977).

Although all of these investigators have studied ponderosa pine as a bioindicator of air pollution impacts, quantification of the effects upon foliage has been limited. For instance, the ongoing studies of Miller *et al.* (1977) in the San Bernardino National Forest demonstrate that air pollution impacts have reduced the growth of 30-year-old ponderosa pine by 83% in areas with the highest ozone concentrations. This study has not yet established several foliar pathologies (tip necrosis, basal scale, basal necrosis, mottle, insect and fungal damage), quantified measurements of needle retention, or determined the concentrations of phytotoxic gases such as sulfur and fluoride in foliage. If these are not quantified for the San Bernardino ponderosa pines in both non-impacted and impacted areas, all measured effects (annual increment growth) could eventually be attributed primarily to oxidants without differentiation from other abiotic agents or natural attrition, all of which can cause one or more of the many foliar pathologies found in pristine and/or polluted areas.

Ponderosa pines in the San Bernardino National Forest are retaining only two years of foliage (Miller *et al.*, 1977), which represents only a 17+ month exposure period from candle stage. This is approximately 43 months less than the average needle retention time of ponderosa pine in southeastern Montana, an area with much less rainfall. Without quantification of foliar pathologies and chemical analysis of different-aged foliage (different exposure time), there is absolutely no way to distinguish between impacts of the different causal agents (phytotoxic gases, acidic precipitation, fungal and insect foliar infestations, and natural abiotic agents such as frost, drought, and mineral deficiencies) in order to determine the relationship between varying chronic concentrations of phytotoxic gases in the ambient air and impacts upon the forest ecosystem.

A good example of the need to quantify baseline sulfur levels in ponderosa pine foliage to determine chronic air pollution impacts is Katz and McCallum's (1939) study in the smelter town of Trail, B.C. They carried out extensive controlled SO_2 fumigation on two conifer species (ponderosa pine and Douglas-fir) in Summerland, B.C., 100 miles west of Trail, B.C., to determine the impacts of different concentrations of SO_2 at varying durations. They reported an average baseline sulfur level (prior to fumigation) of 600 to 700 ppm, with a range of 200 to 1,100 ppm, for the three different-aged foliage (one-, two-, and three-year) of ponderosa pine being tested.

In their field studies around Trail, B.C., and 90 miles south along the Columbia River in Washington, Katz and McCallum reported average sulfur concentrations in the oldest foliage (three-year) ranging from 5,000 ppm (10 miles from Trail) to 1,200 ppm (78 miles from Trail), with an average of 1,500 ppm (90 miles downwind of the smelter). This average level of sulfur 90 miles south of the smelter was more than two times higher than concentrations found in pine foliage from the Summerland, B.C., area. But the fact that foliage pathologies attributable to the air pollution problem were not quantified during the seven-year study allowed the investigators to assume they had a control area in their annual growth increment studies before and after the smelter "abated its emission." If they had carried out needle pathology work to quantify needle retention, tip necrosis, mottle, basal necrosis, and insect and fungal damage, they would have realized that they were actually comparing annual incremental growth of foliage from acute and chronic fumigation zones and that they had no control zone except at Summerland, which they didn't utilize for their control growth studies.

Basically, there is not a single pre- or post-air pollution investigation in the literature today which reports conifer growth/health/damage characteristics as bioindicators of air pollution impacts. Our past and current studies (1976, 1977) in southeastern Montana adequately demonstrate that the damage characteristics of pine foliage, such as needle tip necrosis, mottle, basal necrosis and scale, and insect and fungal damage, occur naturally in pristine environments. Any increase or decrease of these characteristics cannot be ascertained after the advent of air pollution unless they were quantified prior to the impact and unless one recognizes that air pollution damage to pine foliage is mimicked by other causal agents.

We maintain ponderosa pine-skunkbush sites in the immediate vicinity of the two 350 MW coal-fired power plants at Colstrip as well as several miles downwind (80 km) (Figure 5.1). These sites were established on the highest terrain believed to be most likely impacted by the power plant emissions. The first Colstrip unit (350 MW) went on-line in September, 1975, and the second (350 MW) in mid-1976. Since operations began, they have been functioning at various degrees of megawatt capacity; until 1977, neither averaged better than half of their capacity.

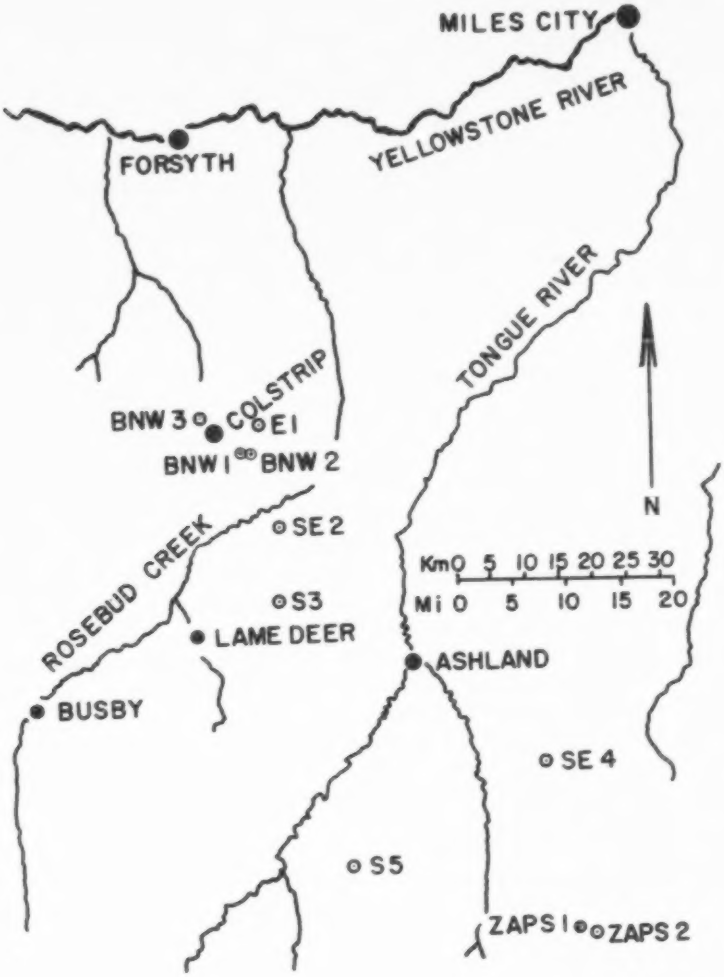

MILES CITY
FORSYTH
YELLOWSTONE RIVER
TONGUE RIVER
COLSTRIP
BNW 3
E1
BNW 1
BNW 2
ROSEBUD CREEK
SE 2
S3
LAME DEER
BUSBY
ASHLAND
N
Km 0 5 10 15 20 25 30
Mi 0 5 10 15 20
SE 4
S5
ZAPS 1
ZAPS 2

Figure 5.1. Ponderosa pine-skunkbush collection sites in the vicinity of Colstrip, Montana

Figure 5.2a depicts the coal consumption of Colstrip Units 1 and 2 during 1976 and 1977, and Figure 5.2b depicts the percent of gross megawatt generation capacity reached by the two units from January to November, 1977. When comparing the coal consumed during the growing season of 1977 (March through September) with that of 1976, it is apparent that more phytotoxic gases probably were emitted during 1977 than during 1976. Unfortunately, the Montana Department of Health and Environmental Sciences (1977) recently declared that its own 1976 and 1977 air monitoring data on SO_2, NO_x, and HF were unreliable; consequently, we do not have usable air quality data from this agency for the early operation periods of the two units.

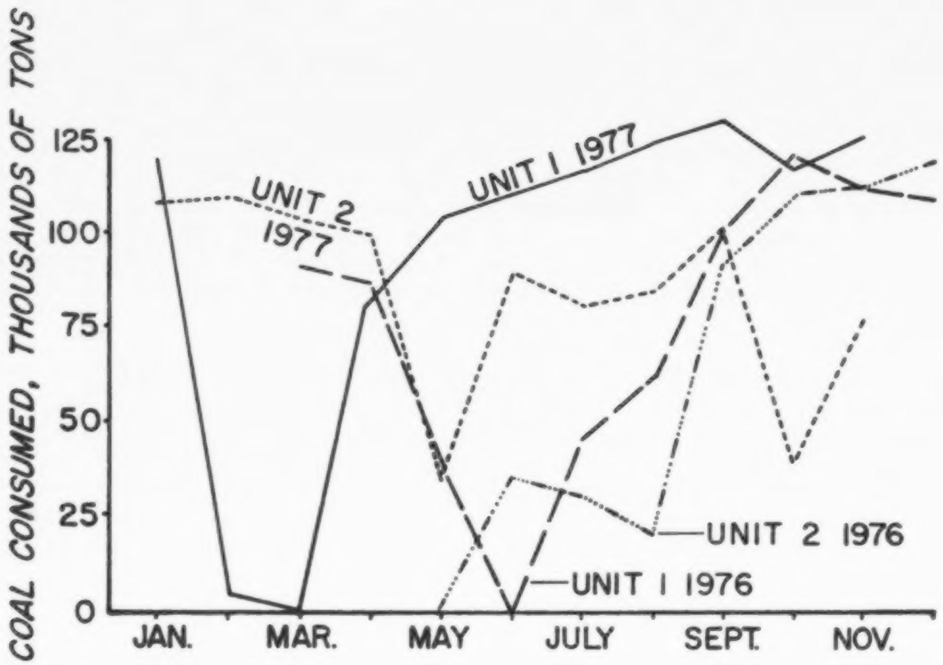


Figure 5.2a. Monthly coal consumption of Colstrip Units 1 and 2 during 1976 and 1977.

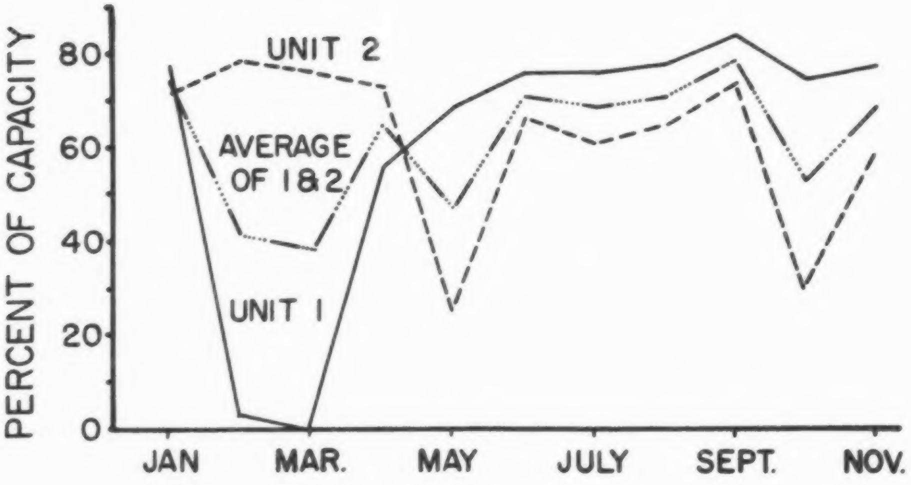


Figure 5.2b. Percent of gross megawatt generation capacity of Colstrip Units 1 and 2 during 1977.

Stack gas and particulate analyses of Colstrip Unit 2 emissions were conducted in March, 1977, by personnel of Battelle Pacific Northwest Laboratories (BPNL) (Crecelius *et al.*, 1978). These studies show that particulates being emitted are extremely small, 80% being less than .5 micron in diameter, and that the scrubbers (wet Venturi) are 99.7% efficient in removing ash from the stack gases. This work also disclosed that the fly ash emissions are enriched in As, Se, Sb, Hg, F, Pb, V, Cu, and Zn, in comparison to concentrations of fly ash retained by the scrubbing system. These more volatile elements are released at a significant rate compared to the less volatile elements.

The Battelle study did not report the rate of SO_2 emissions from the stack. However, data were obtained from personnel of the Montana State Department of Health and Environmental Sciences (DHES) (1978) on stack gas investories compiled by a consulting firm for the Colstrip utility companies. The SO_2 data are from nine stack tests conducted on Unit 2 when it was operating at 327.8 MW per hour capacity. At this capacity, it released between 960 and 1,333 pounds of SO_2 per hour, and assuming an average of 1,146 pounds per hour, both units would collectively release 1¼ tons per hour, 27.5 tons per day, or approximately 10,000 tons per year. This is about 1,000 tons more per year than predicted by the Westinghouse Corporation's Environmental Impact Statement (EIS) in their revision estimates for the Montana State Department of Natural Resources and Conservation (DNRC) hearings in 1976. An emission rate of 10,000 tons of SO_2 is approximately 2½ times less than the annual emissions of the three oil refineries and the 180 MW coal-fired power plant (25,638 tons per year) located within a 15-mile radius of our Billings, Montana, ponderosa pine-skunkbush site GB-1.

The NO_x stack emissions from the two Colstrip units have not been determined at this time, but according to Westinghouse estimates, they should be approximately 20,600 tons per year or 56 tons per day. Westinghouse also predicted that approximately 7,000 pounds of fluoride would be released annually from Units 1 and 2. This amounts to approximately 19.2 pounds per day or .8 pound per hour, approximately 12 pounds per day more than found by either state agencies or by Crecelius *et al.* (1978). This is also five times less fluoride per day (19 vs. 100 pounds) than is emitted by the three oil refineries and the 180 MW coal-fired power plant in the Billings, Montana, area.

The importance of these figures on stack emissions will become apparent later in this section of our Colstrip report in a discussion of the impacts of air pollution at a few of the Colstrip sites and the Billings site where SO_2 and/or fluorides have caused quantifiable effects upon the bioindicator species (ponderosa pine) we are utilizing.

Ambient air monitoring for ozone in the Colstrip area by DHES, EPA, and BPNL disclosed that the average concentration is .04 to .05 ppm, with the highest range being .08 ppm.

In an unpublished annual progress report of the Energy Research and Development Administration (ERDA), Gordon *et al.* (1978b) reported incident rainfall collections in the Colstrip area with pH values ranging from 4.1 to 8.1; some of the lower pH values were not a result of CO_2 dissolved in the precipitation.

Plume strikes during the fall and winter of 1977 at the EPA Hay Coulee site (10 km southeast of Colstrip) were documented by Weber and Olson (1978). Data from continuous analyzers showed instantaneous SO_2 concentrations of 29 pphm. Since this monitoring site is located in a valley, with a high ridge between it and Colstrip, it can be assumed that plume strikes are also occuring at our ponderosa pine-skunkbush sites located on the most elevated ridges in the Colstrip area where only static air monitoring data (sulfation and formate plates) are being gathered.

In this EPA annual report, we are utilizing data collected on growth/health/damage characteristics of ponderosa pine foliage as well as sulfur and fluoride levels in foliage from both EPA sites and four DOE sites. DOE studies are being used because: (1) Three of the sites (BNW-1, BNW-2, and BNW-3) are located in the closest proximity to Colstrip, an area subject to low, chronic air pollution impacts, and the fourth (GB-1) is located in Billings, Montana, an area of elevated chronic air pollution, and (2) 1977 was the last year of funding for the EPA-sponsored study on our ponderosa pine sites, and therefore we would like to demonstrate in this final report the usefulness of various conifer foliage characteristics as bioindicators of chronic air pollution impacts.

METHODS AND MATERIALS

Collection, Measurement, and Evaluation of Ponderosa Pine Foliage

The geographical locations of the five study sites sampled during 1975 and 1976 have been described by Gordon *et al.* (1978a) in the EPA Third Interim Report (hereinafter TIR). Although vegetation was collected at the Whitetail study site (SE-4) during 1975 and 1976, no sampling was done at this site during the 1977 field season because it was felt that further removal of the necessarily large amounts of foliar material required might eventually be detrimental to the trees. Yaeger Butte, located in the Custer National Forest (NW 1/4 of Section 26, T4S, R4SE), was sampled during 1977 as a substitute for SE-4. This site, southeast of Colstrip, was established in 1973 with five trees and expanded in 1975 to ten trees. In addition to Yaeger Butte (hereinafter SE-4), the other sites sampled in 1975, 1976, and 1977 were S-3, S-5, SE-2, and E-1. Foliage was collected only from the upper crown of the trees using the methods detailed in the TIR.

During 1976 and 1977, three sites were sampled in the immediate vicinity of Colstrip, and one site was sampled in Billings, Montana. The sites near Colstrip, BNW-1, BNW-2, and BNW-3, are located 4.5 km SSE, 5 km SE, and 3 km NE of the power plants. The GB-1 site is located 500 meters south of the Montana Power Company's Corette coal-fired steam plant in Billings. Each site is composed of ten permanently-marked trees. The upper crown foliage was collected from the site of the tree facing the emission source.

Using the methods detailed in the TIR, needle length, fascicular cross-sectional area, and percent needle retention were measured. Foliage was evaluated for the presence and extent of those seven pathologies enumerated in the TIR. During 1975, 1976, and 1977, two previously unreported pathologies were also evaluated: percent needles affected by weevil (probably *Scythropos elegans*) (Gordon *et al.*, 1976) and percent needles affected by pathologies not readily categorized. This latter characteristic is called simply "other" pathology and includes necrotic/chlorotic manifestations on needles caused by unidentified parasitic or saprophytic fungi, insect punctures, unclassified abiotic causal agents, or physical damage. This report includes the evaluations of these two pathologies in 1975, 1976 and 1977 collections.

Additionally, the category percent total necrosis was divided into the following components: mottle chlorosis, tip burn necrosis, weevil necrosis, and necrosis and chlorosis resulting from "other" pathologies. Each of these is a visual estimate of the affected portion of the total surface area of 100 needles per internode per tree. THE READER SHOULD BE CAREFUL NOT TO CONFUSE THE CATEGORIES OF MOTTLE CHLOROSIS, TIP BURN NECROSIS, WEEVIL NECROSIS, AND OTHER NECROSIS WITH THE CATEGORIES OF MOTTLE, TIP BURN, TIP NECROSIS, WEEVIL, OR OTHER PATHOLOGIES, RESPECTIVELY, SINCE THE FORMER CATEGORIES REFER TO THE AMOUNT OF LEAF SURFACE OF 100 NEEDLES PER INTERNODE PER TREE (OR 1,000 NEEDLES PER INTERNODE PER SITE) AFFECTED BY THESE INDIVIDUAL PATHOLOGIES, WHILE THE LATTER REFERS TO THE NUMBER (OR PERCENTAGE) OF THESE 1,000 NEEDLES PER INTERNODE PER SITE MANIFESTING THESE PATHOLOGIES.

The needle pathology tip necrosis is also called tip burn in the text of this report. The reason for using both terms to describe the same pathology is that at some of the ponderosa pine sites (GB-1 and BNW sites), this needle pathology is caused by the presence of phytotoxic gases (SO_2, HF) which we call tip burn, while at other sites (SE-4 and S-3) this needle pathology is being caused by such causal agents as drought, frost, or natural attrition. Results are reported herein for the 1975, 1976, and 1977 collections.

Understory vegetation was collected at all study sites during all three years. However, attempts were made during 1977 to collect at least six to eight separate samples of eight to ten species at each site. Of the 19 understory species collected during 1975 and 1976 (TIR), three were not collected during 1977 because of limited availability or identification difficulties: *Aristida longiseta* (red three-awn), *Vicia* spp. (vetch), and *Chrysothamnus nauseosus* (common rubber rabbit-brush).

Chemical Analyses of Vegetation

Methods used to analyze fluoride in vegetation were referenced and summarized in the TIR. The method used for sulfur analysis in vegetation is as follows:

A 0.10 gm aliquot of dried, ground plant material is combusted in an oxygen atmosphere in a Leco induction furnace, and the SO_2 generated is measured by iodometric titration. The amount and concentration of titrant used results in a burette reading calibrated as percent total sulfur for a 1.0 gm sample. For a 0.10 gm sample, the percent sulfur is ten times that indicated by the burette. Antimony metal is placed in the gas delivery tube leading to the titration vessel to mitigate halogen interference, while sodium azide is added to the titration vessel to eliminate nitrogen interference.

Analysis of Vegetation Data

In the TIR, we reported that the results of 1975 and 1976 collections after the data were subjected to analysis of variance (hereinafter anova). Before anova, each derived variate was coded to the arcsine for percentages,* and the coded variates and sample statistics were decoded for the presentation of results. Data for measured variates (sulfur, fluoride, needle length, and area) were not transformed before anova.

Data reported herein for fascicular cross-sectional area, needle length, and sulfur and fluoride levels in pine foliage were first coded to the $\log_{10}$,‡ and the coded variates and sample statistics were decoded for presentation of results. These data were interrogated by three- and four-level nested anova as detailed in the TIR. The following years of foliage origin were used for anova:

1975 collection: 1974, 1973, 1972

1976 collection: 1975, 1974, 1973

1977 collection: 1976, 1975, 1974

Anova was computed on the date from sites S-5, SE-4, E-1, SE-2, and S-3 for the 1975, 1976, and 1977 collections. These results are presented first. Anova was recomputed on data from the latter sites and GB-1, BNW-1, BNW-2, and BNW-3 for the 1976 and 1977 collections. These results are presented next.

Sulfur analyses of 1977 samples are not complete as of this writing and therefore have not been subjected to statistical interpretation; the available data are summarized. Chemical measurements on understory species are also incomplete, and the available data are presented in summary form.

The derived variates (basal necrosis, basal scale, needle retention, healthy needles, mottled needles, tip burn, needles affected by weevil or "other" causes, total necrosis and the chlorosis or necrosis resultant from individual categories) were ranked for graphical presentation of results. For example, Figure 5.3 shows the average ranks for needle retention on 1973 to 1976 foliage from nine sites from the 1977 collection. In the preparation of this figure (and all other figures for derived variates, Figures 5.4 through 5.43), data on each year's foliage from all plots were pooled and ordered from lowest to highest values. Each variate was then assigned a rank (average ranks were computed when ties occurred) so that the lowest and highest values had the lowest and highest ranks, respectively. The ranks were then resorted back into their original samples,* and the average rank ($\frac{\Sigma R}{n}$) was computed for each year of foliage origin at each site. A detailed description of the procedures used to determine average ranks is presented in Appendix 5.1.

---

*coded variate = $\arcsin\sqrt{\frac{\text{variate}}{100}}$

‡coded variate = $\log_{10}$ (variate +1)

No statistically significant differences are implied by any of the figures depicting the average ranks for derived variates. However, the position of the average rank for one sample is relative to that of all other samples. For example, in Figure 5.14, the average rank of tip burn necrosis on 1973 foliage from site SE-4 was decisively lower than the rank of tip burn necrosis on foliage from site E-1. Also, the average ranks depicted segregate the plots into three groups in terms of tip burn necrosis in the 1973 and 1974 foliage. Sites S-5, BNW-2, SE-4, and S-3 had lower average ranks than BNW-1 and E-1; sites GB-1, BNW-3, and SE-2 are higher in average rank than all other sites.

All statistical analyses on derived variates employed non-parametric methods in lieu of single classification anova (Sokal and Rohlf, 1969; Conover, 1971). For treatment effects, the Kurskal-Wallis test was used; for two-sample tests, the Wilcoxon two-sample test was used.

RESULTS

Growth/Health/Damage Characteristics of Pine Needles

1977 Collections Between Sites

When interpreting the data presented in the following graphs, the reader should keep in mind the different ages of foliage and thus the different periods of exposure to the environment. During 1977, pine foliage from the nine sites was sampled from June 25 through mid-September. Because bud break and the candle stage of ponderosa pine usually occur around the first of May in southeastern Montana, the 1976 foliage had been exposed to the environment for approximately 15 to 18 months before being collected. The 1975 foliage had been exposed 27+ months, and the 1974 and 1973 foliage had been exposed for 39+ and 51+ months, respectively. When discussing pathologies and growth characteristics in this report, we will use the approximate exposure periods of 15, 27, 39, and 51 months for different-aged foliage.

Figures 5.3 through 5.6 depict the average ranks of needle retention, percent healthy needles, and two pathologies, mottle and tip burn. Figure 5.3, showing average ranks of needle retention at the nine sites, demonstrates that the characteristic was similar at all sites on 15- and 27-month-old foliage (1976 and 1975). However, between the 27- and 39-month exposure periods, needle retention was substantially reduced at GB-1 in comparison to

---

*A "sample," as used in this context, refers to the ten variates from each year of foliage origin from each plot.

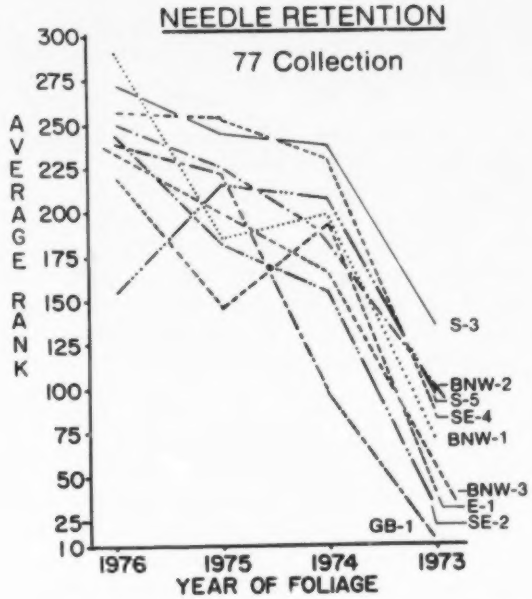


Figure 5.3. Ranks of needle retention, 1977 collection.

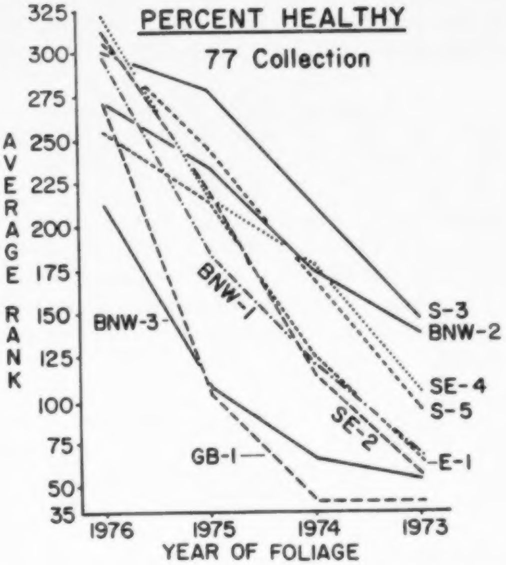


Figure 5.4. Ranks of healthy needles, 1977 collection.

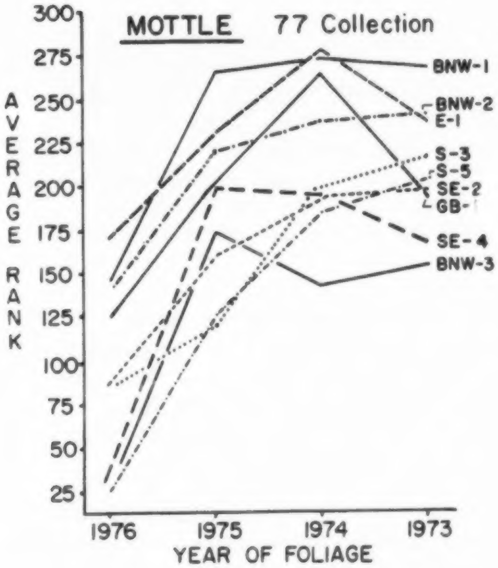


Figure 5.5. Ranks of needle mottle, 1977 collection.

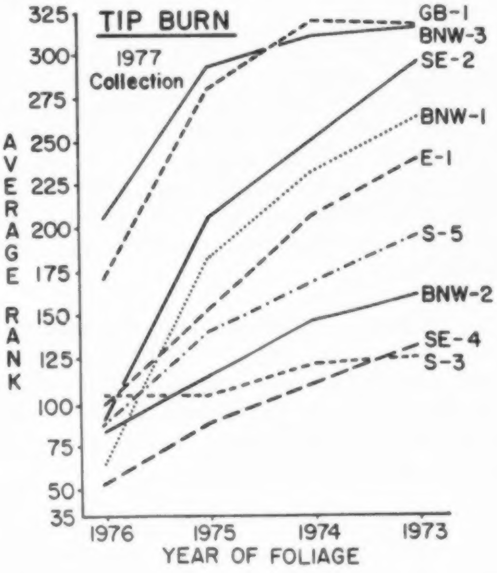


Figure 5.6. Ranks of tip burn, 1977 collection.

the eight Colstrip sites. Needle retention decreased dramatically at all sites during the 39- and 51-month exposure periods (1974 and 1973 foliage) in comparison to any other 12-month period depicted on the graph.

In Figure 5.4, the average ranks of percent healthy needles of different-aged foliage are depicted for the 1977 study period. The data on this figure demonstrate that the percent totally green needles on 51-month old foliage (1973) from the S-3 and BNW-2 Colstrip sites was higher than that found on 27-month foliage (1975) at the GB-1 Billings site and the BNW-3 Colstrip site. Between the 27- and 39-month exposure periods, the percent healthy needles at GB-1 reached essentially zero, while at S-3, SE-4, and S-5, (the more pristine Colstrip sites) and BNW-2 (4.2 km east of the Colstrip power plant), there were still some totally green needles after 51 months exposure.

Figure 5.5 depicts the average ranks of percent needles affected by mottle on different-aged foliage from the 1977 collection period. This pathology is often associated with air pollution studies. However, it was also common on foliage collected in the Colstrip area before the introduction of any industrialization or measurable levels of phytotoxic gases (pre-September, 1975). Between the 15- and 27-month exposure periods, there was a sharp rise in the average rank of needle mottling at all nine sites. But between the 27- and 39-month exposure periods, the percent mottle decreased at BNW-3 and SE-4 and increased slightly at BNW-1 and BNW-2. Between the 39- and 51-month exposure periods, this pathology decreased substantially at both the Colstrip E-1 site and the Billings GB-1 site and was less prevalent on 1973 needles from GB-1, the most polluted of the ponderosa pine-skunkbush sites than on foliage from the most pristine Colstrip site (S-3).

The sharp reduction and the gradual leveling off of mottling between 27- and 51-month-old foliage was evident with a few of the other pathologies on older needles. The reason for this will continually become more evident as the extent of other needle characteristics are reported. However, referring back to Figure 5.3, there is a very dramatic reduction in needle retention between 1975 and 1974 foliage from GB-1 and at all sites between 1974 and 1973 foliage. Needles being cast between the 39- and 51-month periods are believed to have the largest amount of mottling and a few other pathologies.

Figure 5.6 shows the average ranks of tip burn on different-aged foliage during 1977. After 15 months exposure, the amount of tip burn at GB-1 and BNW-3 was substantially higher than that at the other seven sites. By the time the pine foliage had been exposed for 51 months at the nine different sites, there was a large scattering in the average ranks of tip burn between sites.

Figures 5.7 through 5.10 depict two abiotic-caused pathologies, basal scale and basal necrosis, and two insect-caused needle pathologies, defoliator and weevil. Figure 5.7 shows the average ranks of basal scale on different-aged foliage from the nine different sites. The percent basal scale was very similar on 15-month-old foliage from all sites and increased over exposure times (27, 39, and 51 months) at similar rates on all sites.

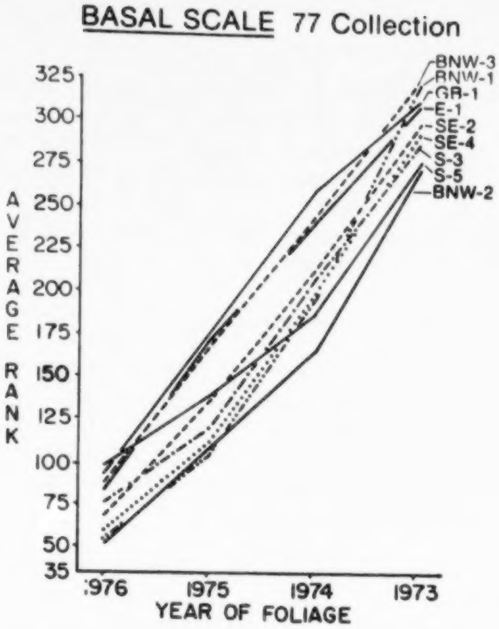


Figure 5.7. Ranks of basal scale, 1977 collection.

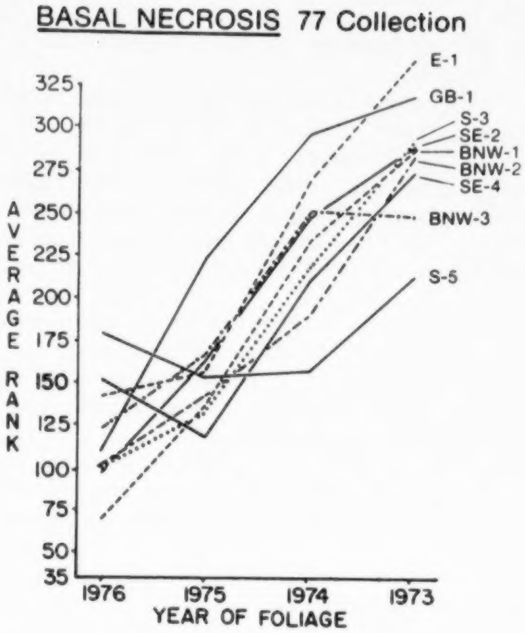


Figure 5.8. Ranks of basal necrosis, 1977 collection.

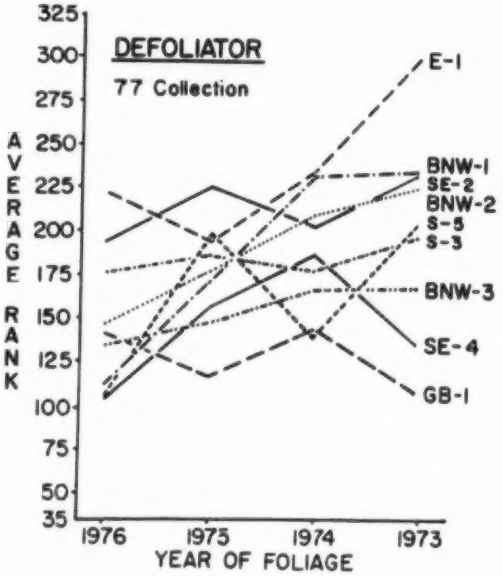


Figure 5.9. Ranks of defoliator, 1977 collection.

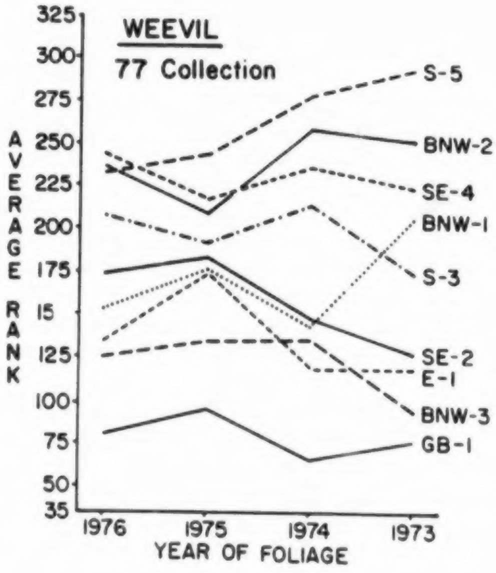


Figure 5.10. Ranks of weevil, 1977 collection.

This pathology was illustrated well in photomicrographs in the TIR and will not be duplicated in this report.

Figure 5.8 shows the average ranks of basal necrosis which increased rapidly over time (15 to 39 months) in foliage collected from GB-1, but began to level off between the 39th and 51st months, as it did at the BNW-3 site. During the last four years, histological and stereoscopic examinations of basal needle tissues manifesting basal necrosis and basal scale have finally led to the conclusion that these two pathologies are caused by abiotic agents (acidic solutions and acidic particulate) and not by insects.

On Figure 5.9 are shown the average ranks of percent defoliator in different-aged foliage from all sites. In comparison to basal scale and basal necrosis over time, defoliator increased substantially at three sites (BNW-1, SE-2, and E-1), and there was very little change in the amount of this insect damage over time at the most polluted sites (GB-1 and BNW-2). There was little to no evidence that weevil damage (Figure 5.10) increased with exposure periods or the age of the foliage. GB-1 was least affected by weevil. This pattern was also evident for defoliator.

Figures 5.11 and 5.12 present two graphs which depict the average ranks of "other" pathologies and total necrosis manifested by different-aged needles at all sites. As previously mentioned, "other" pathologies refers to needle damage caused by saprophytic and parasitic fungi, insect punctures, unidentified abiotic causal factors, and physical damage.

As seen in Figure 5.11, the percent of "other" pathologies increased over time, and this appeared to be similar to the trends observed in basal scale and basal necrosis. Foliage from GB-1 and S-3 had the least amount of "other" pathologies measured in 39- to 51-month-old needles; these two sites are our most polluted and most pristine study sites, respectively.

Figure 5.12 illustrates the amount of total necrosis caused by all needle pathologies at the nine sites during 1977. Total necrosis, the reader is reminded, is the amount of damaged surface area of the needles being retained on their respective internodes. At the GB-1 site, 15-month-old foliage (1976) had the least amount of needle surface damage. Total necrosis was more prevalent on 39-month-exposed needles (1974 foliage) from this site than on either 39- or 51-month foliage from any other site. We believe the drop in rank of total necrosis between the 39- and 51-month-old foliage was due to the casting of the needles most damaged in the older foliage.

The total amount of necrosis/chlorosis which a needle can sustain before being cast during a given time period is not totally understood. Our past three years of study indicate that a needle is cast after 40% to 50% of its surface is damaged or destroyed. During the 1978 growing season, we will attempt to employ a modification of the techniques being used by Miller *et al.* (1977) to collect needles cast from trees in polluted and pristine environments.

Figures 5.13 through 5.16 depict the ranks of the amount of needle surface damage on different-aged needles caused by one or possibly more agents (tip

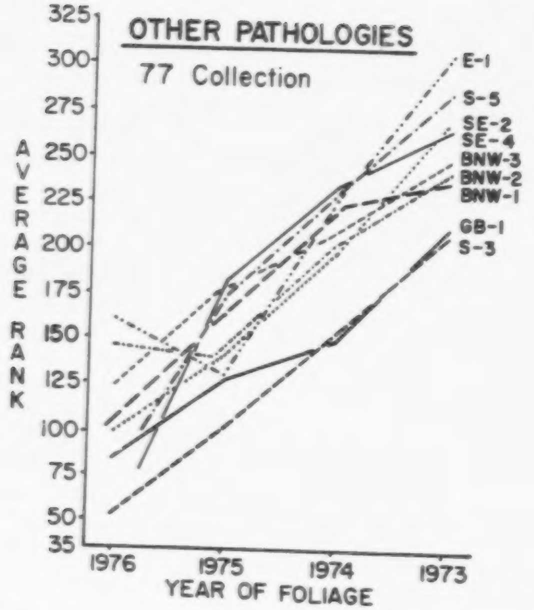


Figure 5.11. Ranks of other pathologies, 1977 collection.

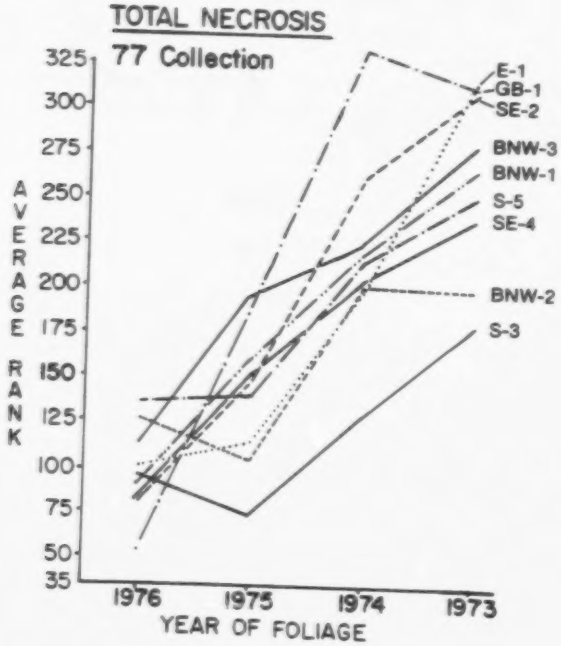


Figure 5.12. Ranks of total necrosis, 1977 collection.

burn and mottle can be caused by such causal agents as air pollution, frost, drought, etc.). Figure 5.13 shows the ranks of the amount of needle surface area affected by mottle chlorosis on different-aged foliage collected and measured in 1977. Over time, the older foliage (1974 and 1973) from GB-1, BNW-1, and E-1 had substantially more needle surface area affected than foliage from other sites. The slight decrease in the amount of mottle chlorosis between 39- and 51-month-old foliage from GB-1 and BNW-1 was more pronounced when the percent of needles manifesting this pathology was ranked (Figure 5.5). However, the general trend was towards a leveling off or a decrease in the prevalence of mottling while the amount of surface area affected remained essentially the same.

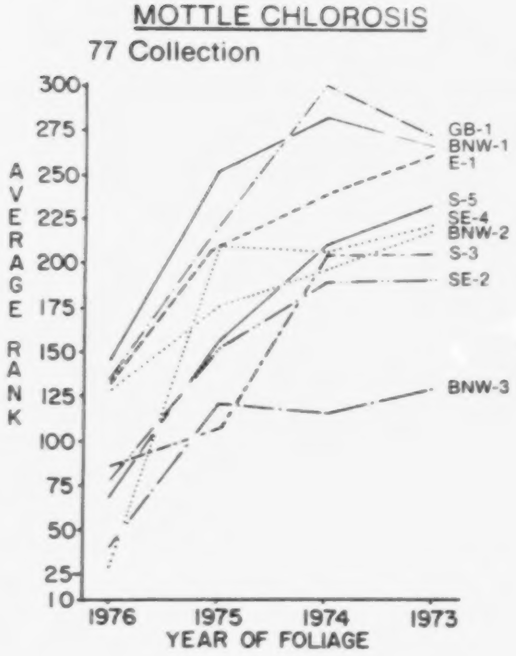
The ranks of needle surfaces manifesting tip burn necrosis are shown in Figure 5.14. In 15-month-old foliage, the amount of needle surface manifesting this pathology was substantially higher at BNW-3 and GB-1 than at the other seven sites. In 27-month-old foliage, it increased dramatically in 15-month-old foliage at BNW-3, GB-1, and SE-2. There was a moderate increase at BNW-1, BNW-2, S-5, and SE-4 and no increase at S-3. The amount of tip burn necrosis leveled off between the 39- and 51-month exposure periods in foliage from GB-1, which was similar to the pattern of mottle chlorosis at this site. It also tended to level off on pine foliage between the 27- and 51-month period at BNW-3, while at BNW-2 it decreased on 1973 needles in comparison to 1974 needles.

The data on tip burn necrosis on 1973 foliage from the 1977 collection at sites GB-1, BNW-3, and SE-2 were subjected to the Kruskal-Wallis test. The Kruskal-Wallis statistic was .633 and was not significant (p > .50). This suggests that the three plots were not significantly different in tip burn necrosis. The Wilcoxon two-sample test was used to compare the sites as pairs and the results of these tests are shown below:

	GB-1	BNW-3
SE-2	44.5	50.0
BNW-3	44.0	

None of the values above are significant (p > .20 for each pair). The distribution of tip burn necrosis at the three sites for 1973 were essentially the same.

The ranked amounts of needle surface damaged by weevil at the different sites are depicted in Figure 5.15, which shows that this endemic insect damage was fairly similar on all-aged foliage within any of the nine sites during 1977. However, there were substantial differences between sites, such as the amount of weevil damage at the pristine S-5 Colstrip site versus that at the polluted GB-1 site.



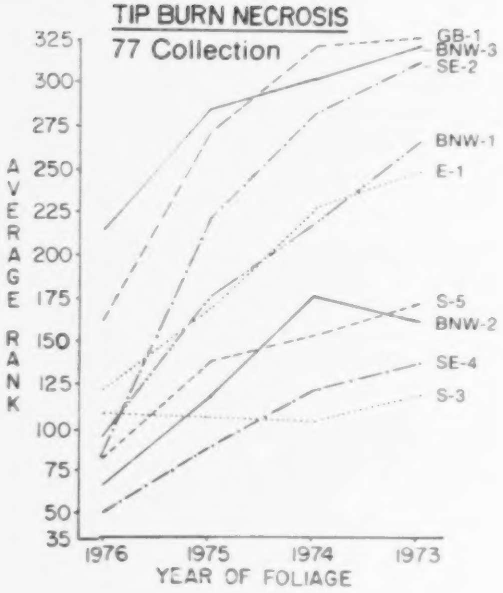
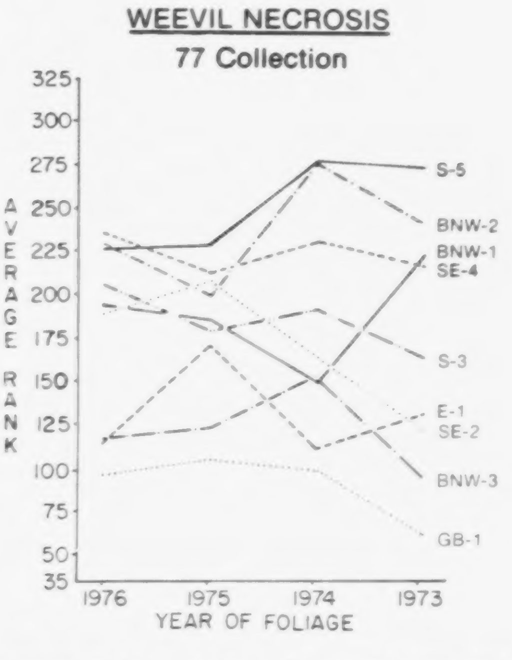


Figure 5.13. Ranks of mottle chlorosis, 1977 collection.

Figure 5.14. Ranks of tip burn necrosis, 1977 collection.



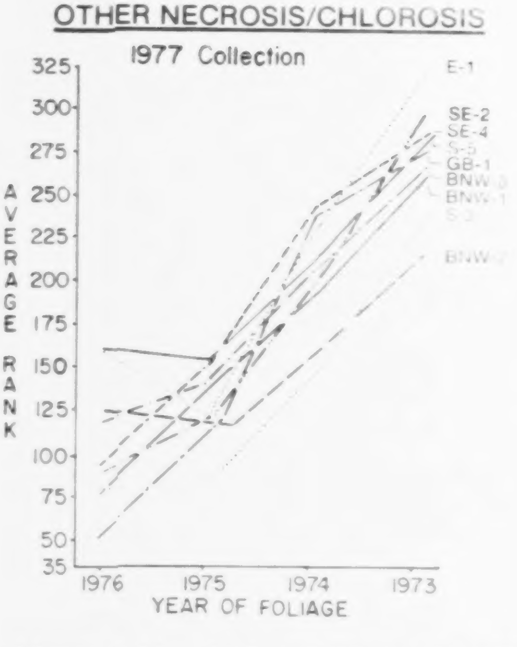


Figure 5.15. Ranks of weevil necrosis, 1977 collection.

Figure 5.16. Ranks of other necrosis/chlorosis, 1977 collection.

Figure 5.16 depicts the ranks of needle necrosis caused by the "other" category of causal agents. The amount of needle area damaged by these causal agents was less at the Billings GB-1 site than at four of the Colstrip sites. However, different-aged foliage from all sites generally displayed similar amounts of damage. This graph demonstrates that foliage from both pristine and polluted sites manifest the various symptoms of the "other" pathology at similar rates.

1977 Collections Within Sites

For the sake of brevity, and because most of the data on growth/health/damage characteristics for ponderosa pine foliage collected at the Colstrip sites is very similar, thus repetitive, we are presenting here the ranks of pathologies for only four sites. These sites were selected because: (1) One is a site where continuous chronic air pollution damage has occurred for several years (GB-1); (2) one is a site in close proximity to the Colstrip power plant (BNW-3, 2.5 km northwest), and (3) two are sites located substantial distances south of Colstrip (S-3, 28 km and S-5, 80 km) and are only impacted occasionally by the plumes of these two power plants.

Figures 5.17 through 5.20 depict the ranks of seven damage characteristics and one health characteristic of foliage collected from the four ponderosa pine sites and analyzed during 1977. Figures 5.17 and 5.18 show the ranks of needle pathologies on foliage from sites S-3 and S-5. At S-3, the most prevalent pathology on 15-month-old foliage (1976) was mottling, and the least prevalent was basal scale. Mottling remained the most prevalent pathology on all-aged foliage, while basal scale became the third most prevalent pathology in 39- and 51-month-old foliage. Tip burn necrosis on all-aged foliage from S-3 remained constant, and it is interesting to note that in 51-month-old foliage from this site, there were more totally green needles than needles damaged by tip necrosis, weevil, or defoliator insects.

At site S-5, mottling of 15-month-old foliage was less prevalent than weevil, basal necrosis, and the "other" category of damage, but at exposure periods of 27 months and more, mottling was surpassed only by the "other" category. As on foliage from S-3, basal scale damage at the S-5 site was the least prevalent on 15-month-old foliage and became the third most prevalent damage on 51-month-old foliage.

If we had categorized both basal needle pathologies as a single needle pathology when we first started the project, this pathology would probably be ranked even higher in prevalence than it appears in our current studies. However, since a single needle base could have both basal scale and basal necrosis and thus be tallied in each of these two columns on the data work sheets, there is currently no way to change our evaluation methods to incorporate these into a single pathology and still allow us to compare them with previous data (1975-1977).

Figure 5.19 shows the average ranks of the seven pathologies on different-aged foliage from site BNW-3. One immediately notes on this graph that percent healthy needles was ranked below all other foliage characteristics during the 27-month exposure period; the previous two graphs demonstrate

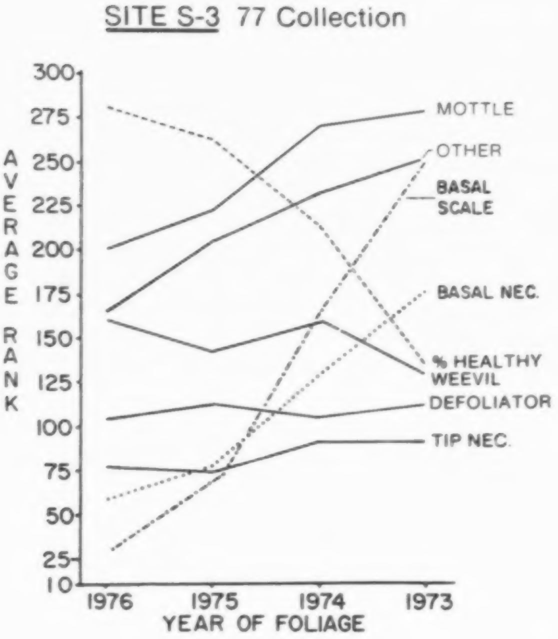


Figure 5.17. Ranks of pathologies at site S-3.

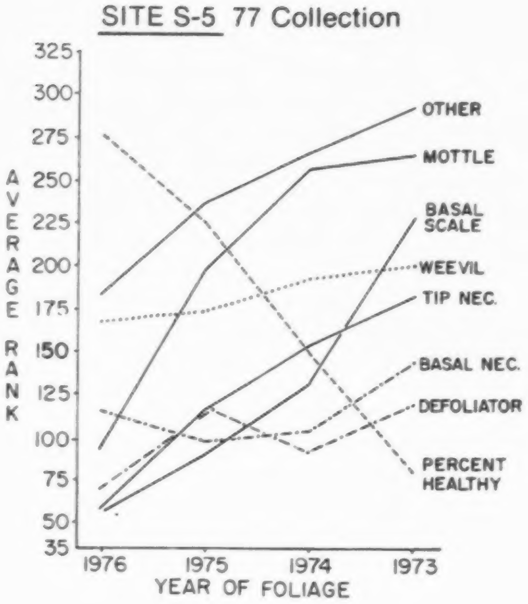


Figure 5.18. Ranks of pathologies at site S-5.

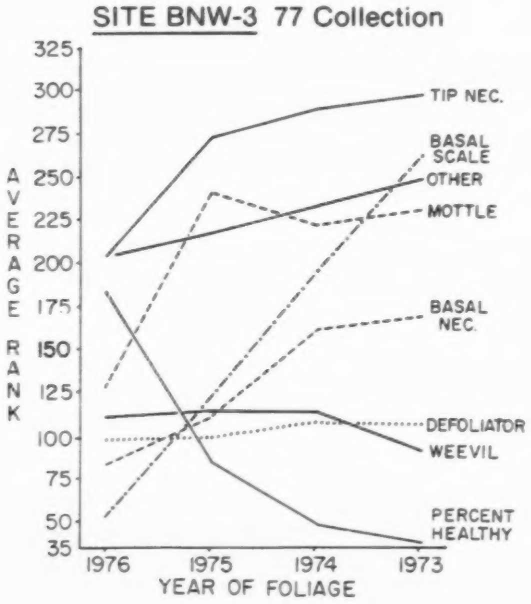


Figure 5.19. Ranks of pathologies at site BNW-3.

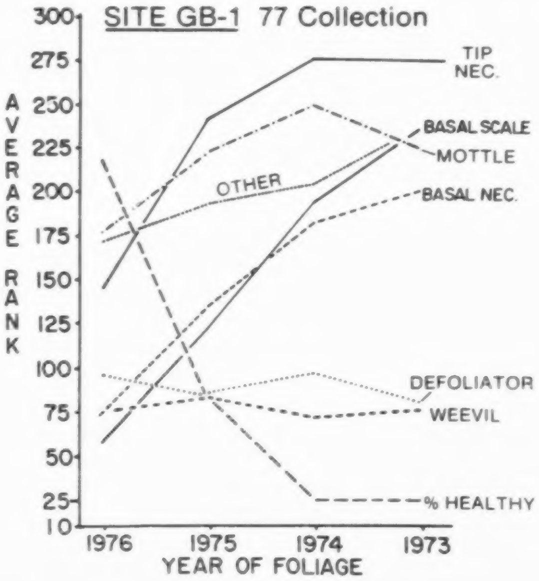


Figure 5.20. Ranks of pathologies at site GB-1.

that this was not the case in S-3 foliage and occurred in foliage from S-5 only between the 39- to 51-month exposure periods.

Tip necrosis and "other" necrosis initially were equally ranked in prevalence at the 15-month exposure period. In all older foliage, tip necrosis continued to be the most prevalent pathology at BNW-3. Mottling increased substantially between the 15- and 27-month period and then decreased in the older foliage (1974 and 1973). Mottling has been used in determining "air pollution impacts" in various studies in the literature. However, the data we have thus far gathered at both pristine (S-3 and S-5) and chronically-polluted sites (GB-1) (see Figure 5.5) on the prevalence of this pathology indicate that the amount of baseline mottling would have to be established before it could be used as an indicator of air pollution impacts.

The ranks of basal scale indicate an initial low prevalence in the 15-month foliage and then, in an almost perfect linear manner, show an increase which surpassed all other pathologies except tip necrosis in the oldest foliage (1973). Both weevil and defoliator remained constant and similar in prevalence in all-aged foliage. As previously illustrated in Figures 5.9 and 5.10, both weevil and defoliator damage at BNW-3 were less prevalent than at most pristine sites, and only foliage from GB-1 was consistently less damaged by these two foliar insects than BNW-3 foliage.

Figure 5.20 illustrates the prevalence of pathologies in foliage collected in 1977 from our most polluted ponderosa pine site, GB-1. After foliage was exposed to the environment of the Billings site for approximately 27 to 39 months, there were no needles that did not manifest some pathology, and thus no healthy needles were recorded. Mottle was slightly more prevalent on 15-month-old foliage than tip necrosis and percent other necrosis, but on the oldest foliage, mottle was ranked as less prevalent than basal scale, tip necrosis, and the "other" necrosis/chlorosis category. In the next set of figures, one will note that not only was mottling less prevalent on these older needles (1973), but less needle surface area was affected.

The pattern for the prevalence of defoliator and weevil damage in the various-aged foliage from the GB-1 site was very similar to that observed on foliage from the BNW-3 site (Figure 5.19).

Figures 5.21 through 5.24 depict the pathologies which caused the most and least prevalent amounts of needle surface damage. Rather than discuss each graph separately, it is more illustrative to discuss all four together, although the different pathologies are not ranked here between sites but within each site. The ranks of tip necrosis damage to needle tissue within all four sites on Figures 5.21 through 5.24 adequately demonstrate that this pathology was the major manifestation of tissue damage on pine foliage from BNW-3 and GB-1 but was responsible for the least amount of damage on foliage from S-3 and S-5. At these latter sites, the unidentified abiotic and biotic causal agents of the "other" pathology were more prevalent than any other pathology on 51-month-old foliage. The "other" necrosis/chlorosis category at BNW-3 and GB-1 was also ranked higher than mottle and weevil damage on the

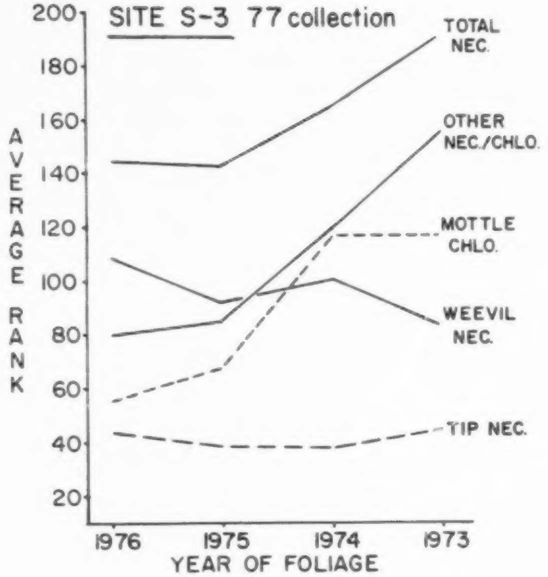


Figure 5.21. Ranks of needle damage at site S-3.

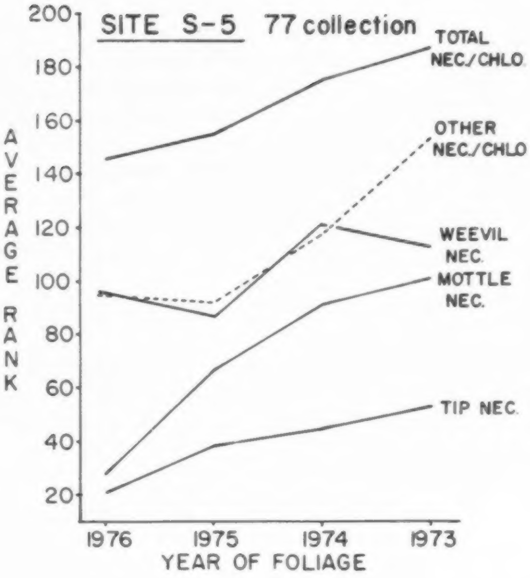


Figure 5.22. Ranks of needle damage at site S-5.

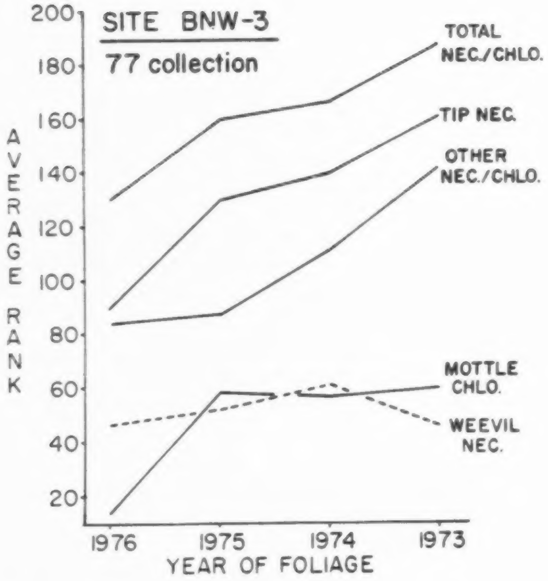


Figure 5.23. Ranks of needle damage at site BNW-3.

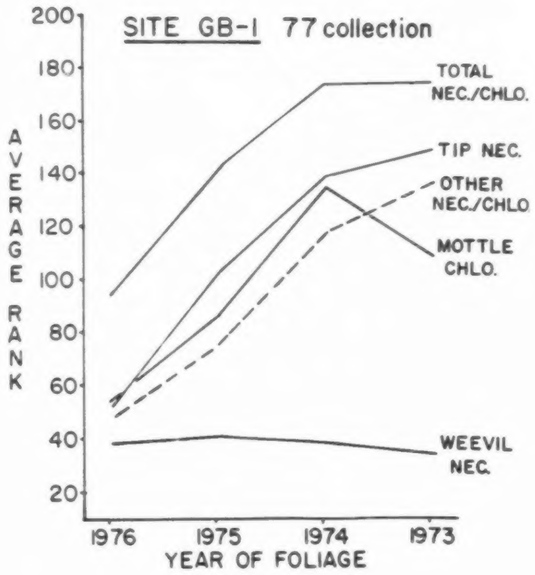


Figure 5.24. Ranks of needle damage at site GB-1.

51-month foliage. The amount of needle tissue being damaged by pine weevil at GB-1 and BNW-3 was ranked very low in comparison to other types of pathologies, with the exception of mottling at BNW-3. One notes on Figure 5.19, that mottling was ranked substantially higher than weevil on the 27- to 51-month-old foliage from this site. After comparing the ranks of mottling with weevil damage on Figure 5.19 and then comparing them on Figure 5.23, it is apparent that one pathology can be substantially more prevalent than another (Figure 5.19) but not necessarily cause more tissue damage (Figure 5.23).

All Collections of 1973 and 1974 Needle Between Sites

The next series of graphs show the average ranks of growth/health/damage characteristics of 1973 and 1974 needles as they were quantified during each collecting and study period of 1975, 1976, and 1977 at the Colstrip sites only. Pathologies on trees from the Colstrip BNW sites were not evaluated until the spring of 1976.

When interpreting the patterns of different pathologies on 1973 and 1974 needles over the three-year period, the reader should keep in mind the amount of time of exposure of the foliage. In 1975, for instance, the 1974 needles had been exposed to their environment for approximately 15 months and 1973 needles for approximately 27 months before being collected. In 1977, the 1974 needles had been exposed for approximately 39 months and 1973 needles for approximately 51 months.

Figures 5.25 through 5.38 illustrate the rank of needle retention, percent healthy needles, tip necrosis, and percent mottle of 1973 and 1974 foliage over the last three years.

Figure 5.25, which depicts the average ranks of needle retention at the five Colstrip sites, shows that 1973 foliage from SE-2 and E-1 was cast faster during all exposure periods (27 to 51 months) than at S-3. This same trend was evident in 1974 foliage from E-1 and SE-2 (15 to 39 months). The order of average ranks of needle retention on 51-month foliage (1977 collection of 1973 needles) from all five states was almost identical to the rank sequence for 1974 foliage at the 39-month exposure period (1977). As one studies the ranks of needle pathologies in the following graphs, it should be noted that foliage from SE-2 and E-1 usually rank higher in most pathologies than foliage from the other three sites. Needle casting is a continuous phenomenon over time even in "pristine areas" such as the Custer National Forest. It is essential to determine which needles are being prematurely cast and why before the impacts of chronic air pollution to coniferous forest species can be understood.

Figure 5.26 depicts the average ranks of percent healthy 1973 and 1974 foliage. The almost linear loss of totally green needles being retained on 1973 and 1974 internodes over time demonstrates that foliar exposure periods takes its toll on the chlorophyllic food-producing tissues, even in "pristine" areas. In general, the rank sequence of 51-month (1973) foliage and 39-month (1974) foliage on the five sites was similar to that of needle retention.

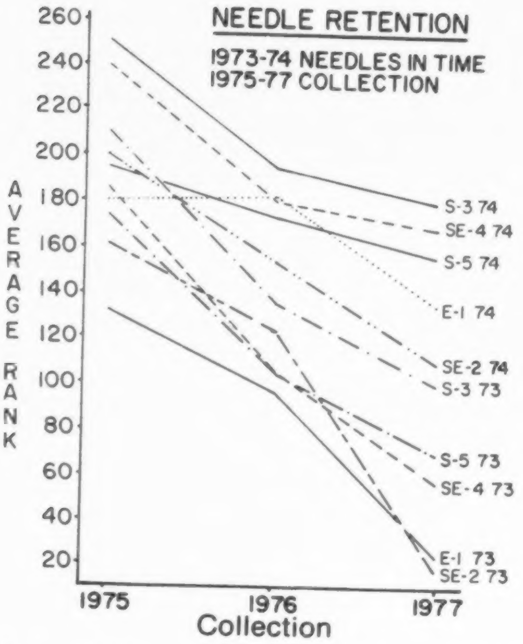


Figure 5.25. Ranks of needle retention, 1975-77 collection.

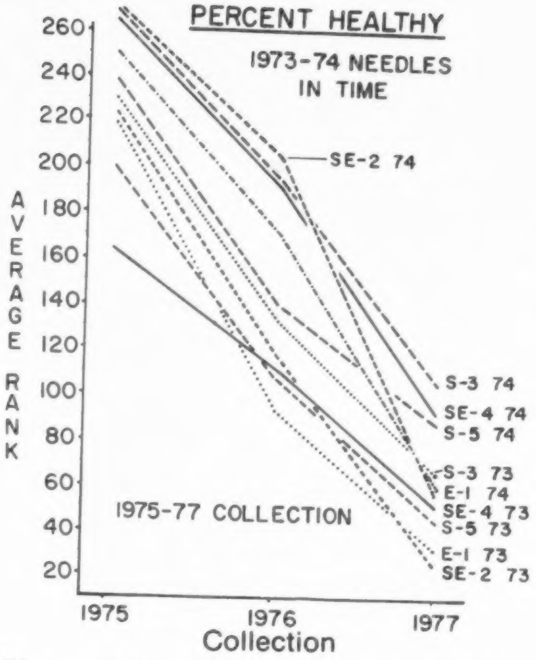


Figure 5.26. Ranks of healthy needles, 1975-77 collection.

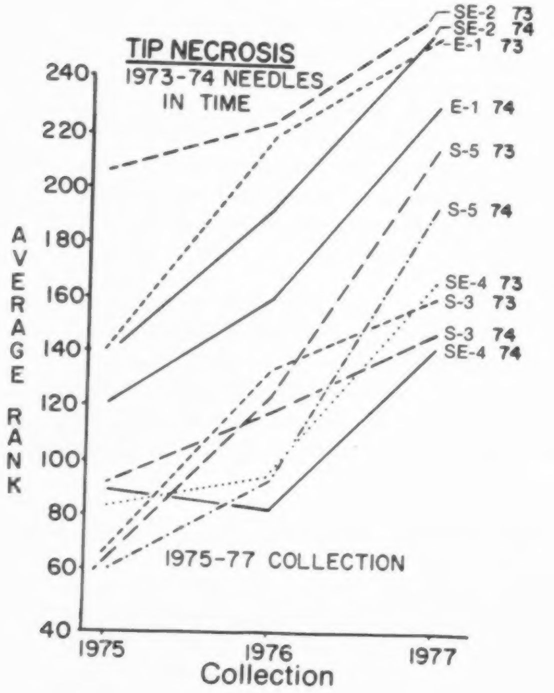


Figure 5.27. Ranks of tip necrosis, 1975-77 collection.

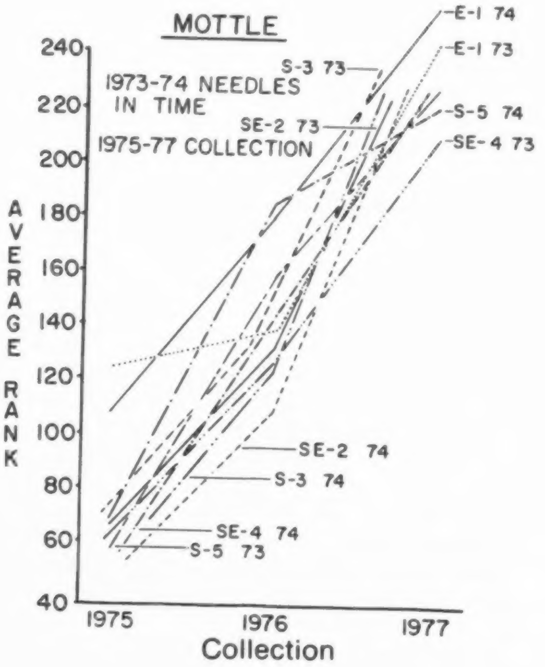


Figure 5.28. Ranks of needle mottle, 1975-77 collection.

Figure 5.27 illustrates the average ranks of tip necrosis on 1973 and 1974 needles over time. The 1973 foliage from SE-2 had a higher percentage of tip necrosis at all exposure periods than occurred at other sites except E-1. The 1973 and 1974 foliage from S-3 and S-4, after 51- and 39-month exposure periods, respectively, had substantially less tip necrosis than foliage from other sites.

The occurrence and increase in mottling (Figure 5.28) over time on 1973 and 1974 foliage were extremely similar at all sites. While there were substantial differences in prevalence between exposure periods (15 vs. 39 or 27 vs. 51 months), differences during the same exposure period were slight.

Figures 5.29 through 5.32 depict the average ranks of basal scale, basal necrosis, weevil, and defoliator damage to 1973 and 1974 needles over time. As demonstrated in Figure 5.29, the rank of basal scale leveled off in the 39-month-old foliage (1973) from three of the pine sites (S-5, S-3, and E-1). This means that either the increase in this pathology abruptly ceased on this year's foliage between the 39- and 51-month exposure periods or that the needles being cast from the 1973 internodes were those with basal scale. The substantial difference in rank between the 1973 and 1974 foliage over time demonstrates, or strongly suggests, that this pathology is directly related to exposure time at these pristine pine sites.

The average ranks of basal scale are depicted in Figure 5.30. One notes when studying the data on this graph that both 1973 and 1974 foliage collected in 1977 from S-5 had substantially less basal necrosis than was evident during the 1976 study period. Foliage from this site also showed the largest increase of basal necrosis between the 1975 and 1976 collection periods. Figure 5.32 demonstrates that the rank of percent defoliator on 1973 and 1974 foliage from S-5 also dropped substantially in prevalence between the 1976 and 1977 study periods. Thus, these two types of damage would seem to have been very prevalent on both years foliage which were prematurely cast from the internodes during this time.

Figure 5.31 depicts the ranks of weevil damage at the five Colstrip pine sites. Probably the trend most evident in this graph is the similarity of weevil damage prevalence on 1973 and 1974 needles within a site but not between sites. We do not know why this prevalence decreased slightly at most sites on both-aged foliage during 1976, but the phenomenon could have been partially caused by measurable differences of rainfall between 1976 and 1977 in southeastern Montana.

Trends in the prevalence of defoliator damage (Figure 5.32) on both years foliage from within plots, such as SE-2, E-1, and S-5, were similar to patterns observed for weevil damage (Figure 5.31). However, defoliator prevalence within plots on 1973 and 1974 needles over time was not evident at sites S-3 and SE-4. Probably the most obvious pattern of insect damage on 1973 and 1974 needles, as depicted for both weevil (Figure 5.31) and defoliator (Figure 5.32), was that they did not increase continuously and/or substantially over time (15-51 months), as did the other pathologies such as basal scale, basal necrosis, and tip necrosis.

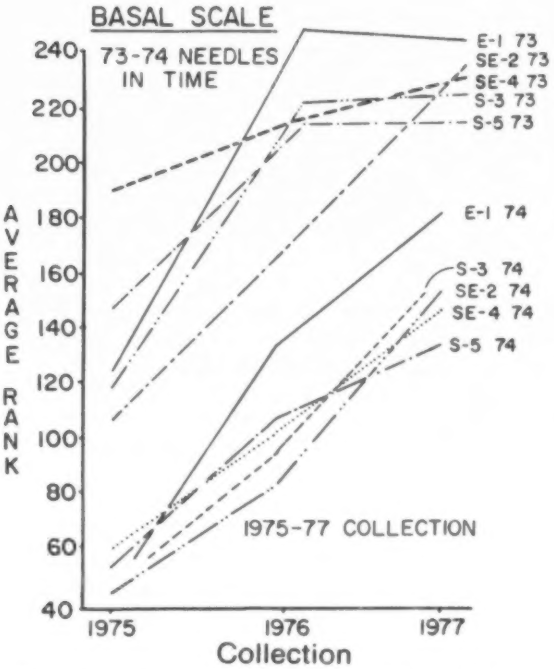


Figure 5.29. Ranks of basal scale, 1975-77 collection.

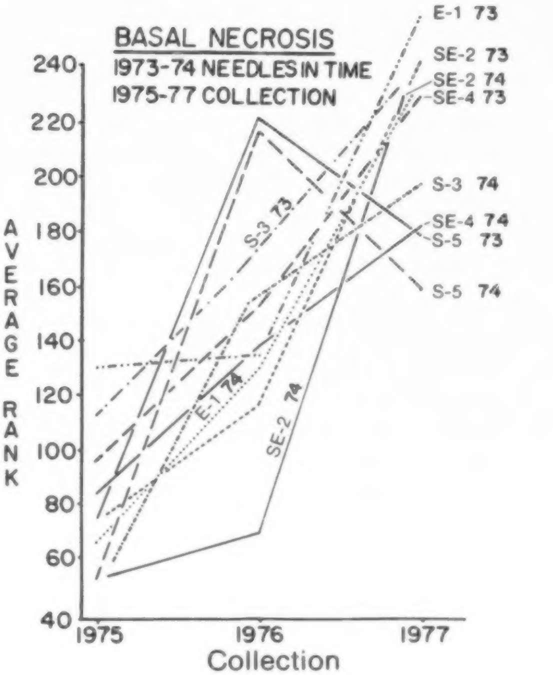


Figure 5.30. Ranks of basal necrosis, 1975-77 collection.

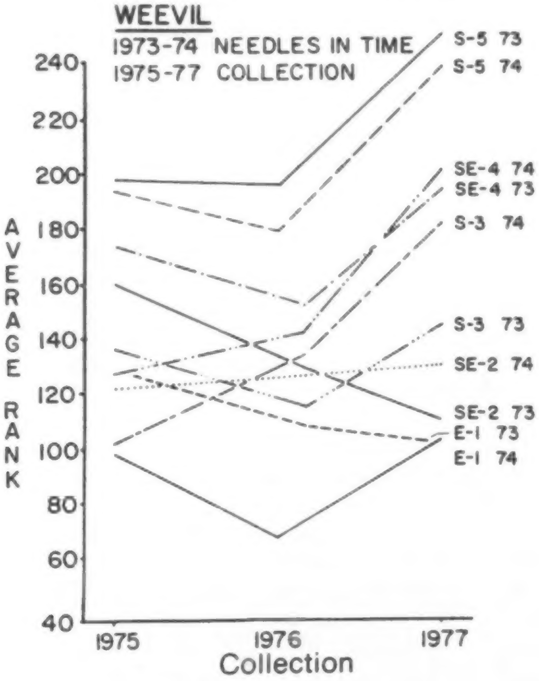


Figure 5.31. Ranks of weevil, 1975-77 collection.

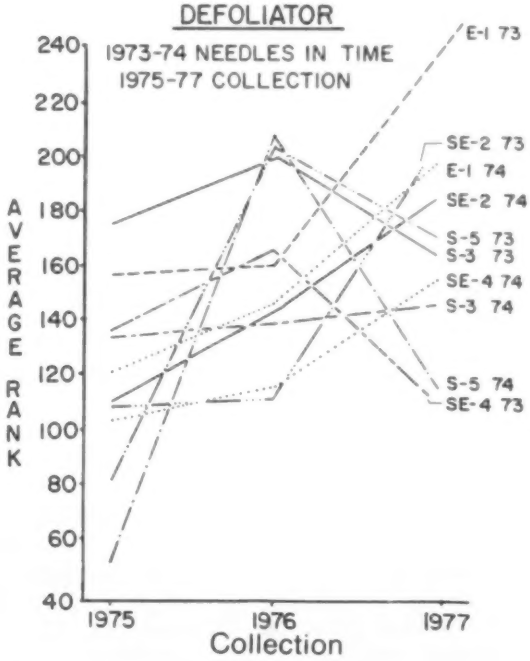


Figure 5.32. Ranks of defoliator, 1975-77 collection.

Figure 5.33 illustrates the ranks of needle pathologies in the "other" pathology category at the five Colstrip sites during the 1974 through 1977 period; Figures 5.34 through 5.36 depict the growth/health characteristics of 1973 and 1974 pine foliage collected during 1976 and 1977 at all nine sites. Trends of the "other" pathology on 1973 and 1974 foliage at the Colstrip sites were very similar to those of mottling (Figure 5.28). There was a continuous increase in prevalence as the 1973 and 1974 needles were exposed for longer periods.

To demonstrate how the five Colstrip sites compared with the three BNW sites and the Billings site, growth/health/damage characteristics of 1973 and 1974 foliage during the 1976 and 1977 collecting periods have been ranked for all nine sites. Again, it should be noted that 1973 needles collected during 1976 were approximately 39 months old, and the 1974 foliage was approximately 27 months old. Therefore, in 1977, the 1973 and 1974 needles were approximately 51- and 39-months-old, respectively.

Figure 5.34 illustrates the ranks of needle retention at all nine sites during the 1976 and 1977 collection periods. Needle retention on 1973 foliage was less prevalent at GB-1 than at all other sites, and 1974 foliage (39-month exposure period) from this site displayed less needle retention during 1977 than 51-month (1973) foliage at S-5 and S-3. If one looks back at Figure 5.25, the addition of the three BNW sites and GB-1 to the rank system reduced the slope of needle retention for the five Colstrip sites.

Figure 5.35 depicts the average ranks of the prevalence of percent healthy 1973 and 1974 needles during the 1976 and 1977 collection periods. Both 1973 and 1974 foliage from GB-1 and BNW-3, exposed for 27 to 51 months, had substantially less totally green needles than foliage from the other sites, except 51-month-old foliage (1973) from SE-2 and E-1. Also, both 1973 and 1974 foliage from BNW-2 had more totally green needles than foliage from the other Colstrip sites.

Figure 5.36 illustrates the ranks of tip burn on 1973 and 1974 foliage during 1976 and 1977. This pathology was more prevalent at GB-1 and the BNW-1 sites than any of the Colstrip sites. One notes that there was no change in the prevalence of tip necrosis on 1973 needles from GB-1 between 1976 and 1977. Discussion of the tests for significance between the amount of tip burn on foliage from GB-1, BNW-3, and SE-2 was presented earlier.

Figure 5.37 presents the ranks of mottle which occurred on 1973 and 1974 foliage during 1976 and 1977. It is immediately apparent that this pattern at both GB-1 and BNW-3 was opposite that seen at other ponderosa pine sites. The importance of this trend of reduced needle mottling over time in chronically-polluted areas cannot be overestimated. Returning to Figure 5.5, one notes that percent mottle increased between 1974 and 1975 foliage from GB-1. However, its prevalence on 1974 foliage actually decreased between the 1976 and 1977 collection periods because of premature needle casting. Thus, while a single year's study on damage characteristics can provide substantial information, it does not allow an investigator to interpret the trends of the prevalence of certain pathologies over time at sites such as BNW-3 and GB-1.

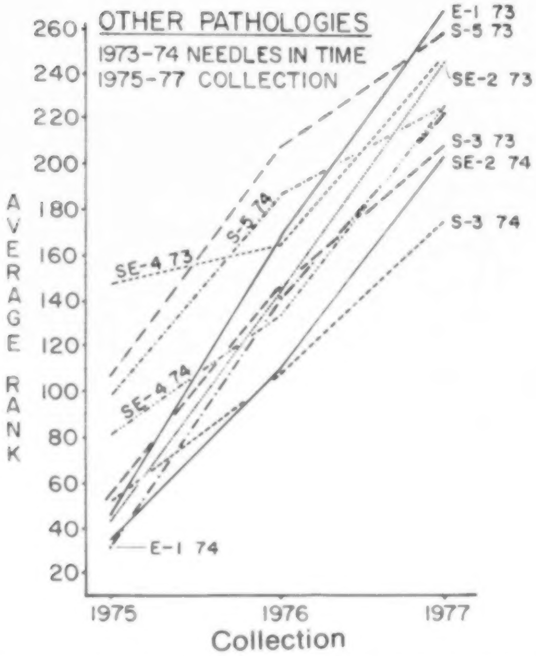


Figure 5.33. Ranks of other pathologies, 1975-77 collection.

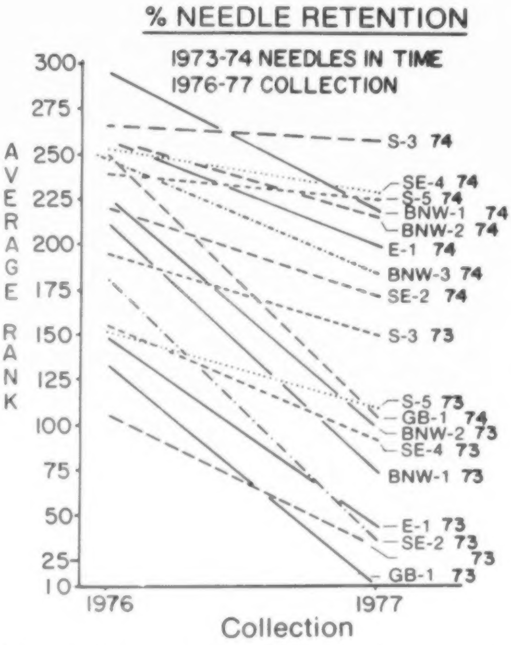


Figure 5.34. Ranks of needle retention, 1976-77 collection.

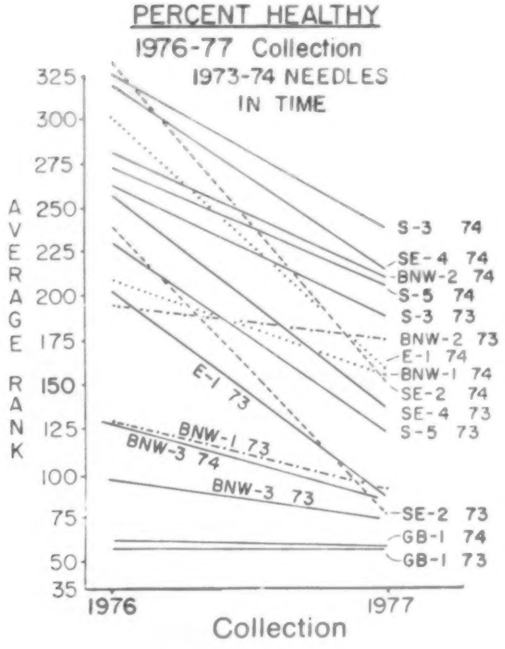


Figure 5.35. Ranks of healthy needles, 1976-77 collection.

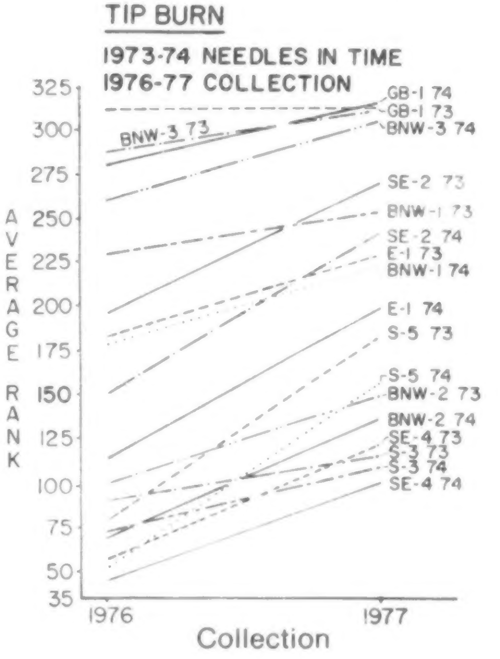


Figure 5.36. Ranks of tip burn, 1976-77 collection.

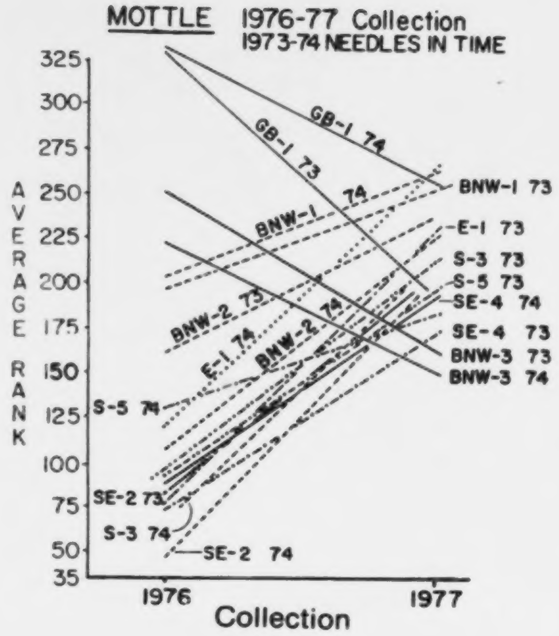


Figure 5.37. Ranks of needle mottle, 1976-77 collection.

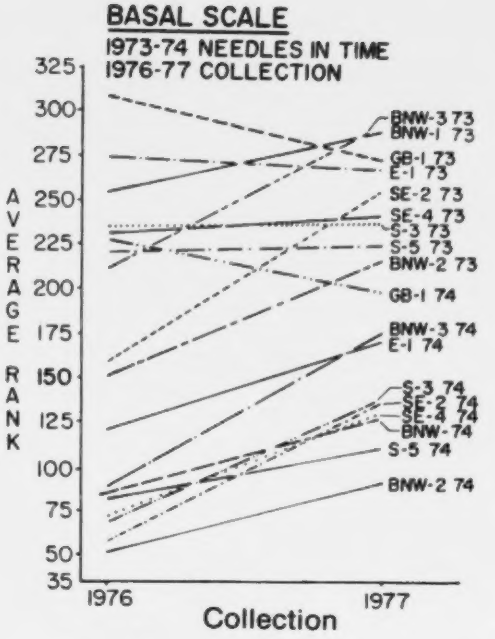


Figure 5.38. Ranks of basal scale, 1976-77 collection.

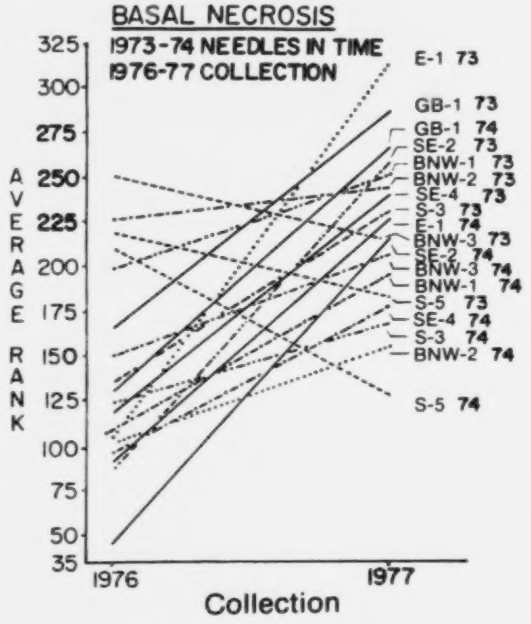


Figure 5.39. Ranks of basal necrosis, 1976-77 collection.

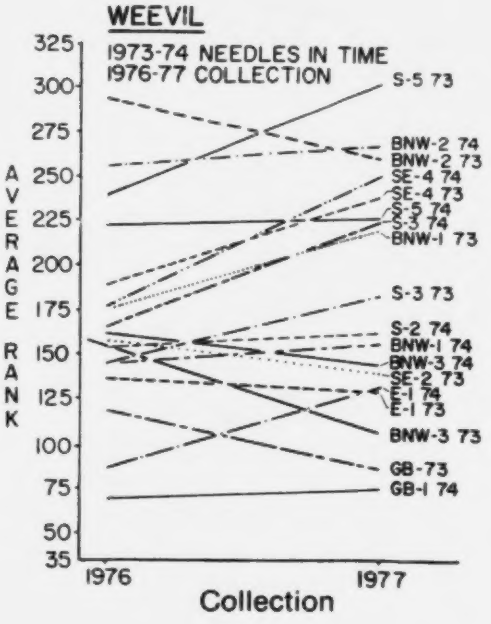


Figure 5.40. Ranks of weevil, 1976-77 collection.

Figure 5.38 depicts the ranks of basal scale on 1973 and 1974 foliage from the nine sites during 1976 and 1977. At E-1, S-3, S-5, SE-4, and GB-1, basal scale tended to level off or decrease on 1973 foliage between the 1976 and 1977 collections. Only at GB-1 was there a decrease in the prevalence of basal scale on 1974 foliage during these two collection periods. Turning back to Figure 5.29, one sees the trend for basal scale in 1973 and 1974 needles from the five Colstrip sites, but in Figure 5.7, this trend is not evident from a single year's collection or analysis of different-aged foliage.

Figure 5.39 depicts the ranks of basal necrosis on 1973 and 1974 foliage over the 27- to 51-month exposure periods. At S-5, there was a decrease in this pathology on both 1973 and 1974 foliage during the two collection periods. It also decreased on 1973 foliage at BNW-3 during this time. The same trend is more discernible in the data on Figure 5.30 but was not evident at site S-5 as shown on Figures 5.8 and 5.10.

Figure 5.40 illustrates the prevalence of weevil damage on 1973 and 1974 foliage over time. Probably the most interesting aspect of the graph is that it confirms the trends for this needle pathology on foliage from the GB-1 and BNW-3 sites depicted in Figure 5.15. The fact that we found less insect damage at GB-1 is supported by the rank of defoliator damage on Figure 5.41 as well as the rank of the "other" pathology category on Figure 5.42.

The rank of defoliator damage on 1973 and 1974 foliage (Figure 5.41) from S-5 is of interest because it might be inferred from Figure 5.9 that the percent of this pathology on 1973 foliage increased between the 39- and 51-month (1974 and 1973) exposure periods. However, Figure 5.41 shows that there was an almost identical amount of defoliator damage on 1973 and 1974 foliage in 1976 and that there was a substantially larger decrease of defoliator damage in 1974 needles than in 1973 needles. Thus it would be more correct to infer that the needles cast from 1974 internodes had more defoliator insect damage than those being cast from 1973 internodes at this site during the 12-month period.

Figure 5.43 illustrates the ranks of percent total necrosis on 1973 and 1974 needles during 1976 and 1977. Turning back to Figure 5.12, again one can ascertain the importance of comparing damage pathologies over time versus a single collection period. As previously stated in this text, we have found that when total necrosis affects between 40% and 50% of a needle surface, that needle will probably be cast from the internode. Most of the figures on needle pathologies from the 1977 collection previously discussed in this section demonstrate some increase of needle pathologies from younger needles (1975-1976) to older needles (1973-1974). Figure 5.3 shows that needles are being cast continuously at varying rates on all internodes studied.

As depicted in Figure 5.43, the percent of total necrosis on 1973 and 1974 needle surface areas from GB-1 remained constant during 1976 and 1977, although there was substantial needle casting during this two-year period (Figure 5.34). This same trend was also apparent on 1973 and 1974 needles

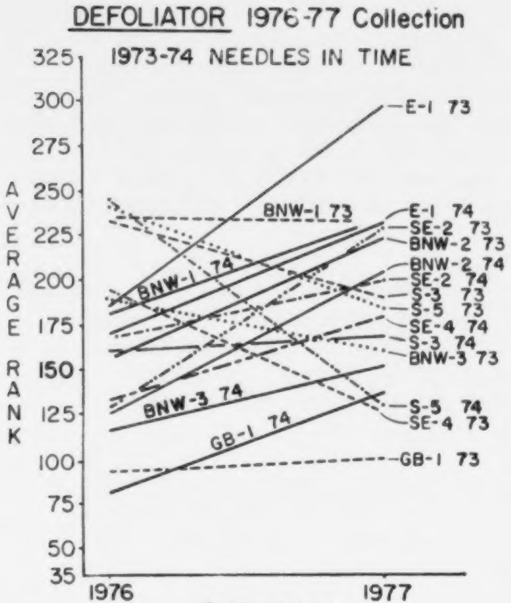


Figure 5.41. Ranks of defoliator, 1976-77 collection.

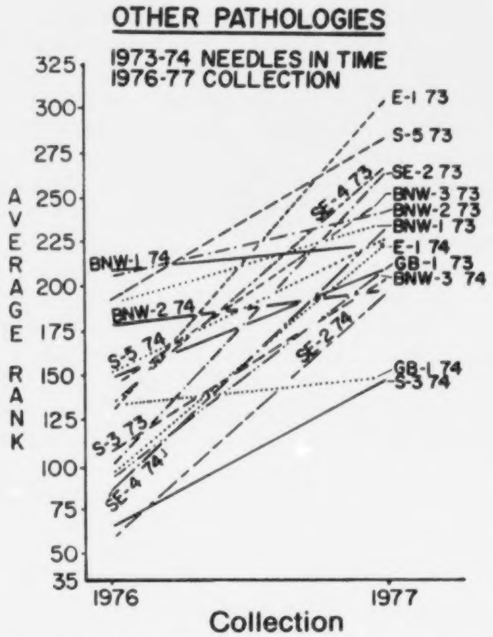


Figure 5.42. Ranks of other pathologies, 1976-77 collection.

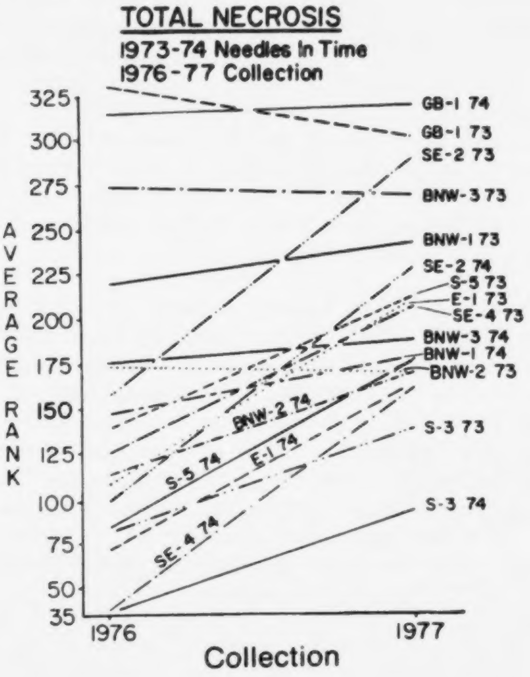


Figure 5.43. Ranks of total necrosis, 1976-77 collection.

from BNW-3 and on 1973 needles from BNW-1 during 1976 and 1977 collection periods. The fact that the amount of necrosis on 1973 and 1974 foliage did not change between the 27-month (1974 needles collection in 1976) and 51-month (1973 needles collected in 1977) exposure periods strongly suggest that there is a very quantifiable limit of total needle surface which can be damaged prior to being prematurely cast. As illustrated in Figures 5.52 and 5.62, the mean fluoride and sulfur levels in different-aged foliage collected from GB-1 tend to level out rather than increase in foliage. We suspect that the damaged needles being prematurely cast from the pine trees at the GB-1 site are not only the most damaged, thus the leveling trend seen in Figure 5.43, but have the highest fluoride and sulfur concentration. This hypothesis will be discussed in some detail in the following section.

Fluoride, Needle Length, and Fascicular Cross-Sectional Area

Because it was necessary to substitute the Yaeger Butte site, SE-4, during 1977 collections for the Whitetail site sampled during 1976, 1977 data from SE-4 was used in three-level nested anova on 1975 and 1976 collections. F ratios for indicated levels for 1975, 1976, and 1977 collections are shown in Table 5.1. F ratios in parentheses in Table 5.1 are the values reported in the TIR for the appropriate level. As may be seen in this table, substitution of the Yaeger Butte site had minimal influence on significant treatment effects; in the 1975 collection, a significant treatment effect for cross-sectional area between the tree ages was shown. A treatment effect ($p = .0566$) was seen for needle length in the 1976 collection between tree ages. Significant effects at these levels were not detected with the Whitetail site (Table 5.1).

Figure 5.44 depicts the mean values and 95% confidence intervals for fascicular cross-sectional area of upper crown foliage, younger and older trees, for all sites in the 1975 collection. The mean values between younger and older trees within site SE-4 were significantly different at $p \leq .05$.

Figure 5.45 depicts the mean values and 95% confidence intervals for needle length at all sites in the 1976 collection, upper crown, for younger and older trees. The mean values for S-3 were significantly different at $p \leq .02$.

Table 5.1 also shows F ratios for the indicated levels for anova computed on the data from the 1977 collection. Significant treatment effects ($p \leq .05$) were detected between sites for needle length, while a treatment effect between years of foliage within tree ages was detected for fascicular cross-sectional area.

Figure 5.46 depicts the mean values and 95% confidence intervals for fascicular cross-sectional area of 1974, 1975, and 1976 upper crown foliage from all plots in the 1977 collection. Mean values for cross-sectional areas (Figure 5.46) for 1975 foliage were smaller than those for 1974. Although these relative positions (1975 < 1974) were also observed during 1976, the cross-sectional areas of 1973 foliage were slightly greater than those of 1974. (For illustration, the mean values and 95% confidence intervals for 1973 foliage from the 1976 collection are shown in Figure 5.46

TABLE 5.1. F RATIOS FOR FLUORIDE, NEEDLE LENGTH, AND FASCICULAR CROSS-SECTIONAL AREA FOR THE THREE-LEVEL NESTED ANOVAS FOR THE 1975, 1976, and 1977 COLLECTIONS

Level	Fluoride	Needle Length	Fascicular Cross-Sectional Area
1975 Collection Variable			
Between Sites	24.988* (9.144)*	5.664* (10.480)*	5.206* (5.660)*
Between Tree Ages Within Sites	.249 (1.939)	1.452 (1.217)	2.944* (1.348)
Between Years of Foliage Within Tree Ages	1.253 (.751)	1.313 (1.374)	.496 (.500)
1976 Collection Variable			
Between Sites	2.757 (2.025)	7.289* (10.286)*	6.754* (6.629)*
Between Tree Ages Within Sites	1.761 (1.532)	2.604 (1.767)	1.170 (1.265)
Between Years of Foliage Within Tree Ages	.680 (.819)	.700 (.858)	.945 (.979)
1977 Collection Variable			
Between Sites	4.784[‡]	31.269*	2.306
Between Tree Ages Within Sites	1.037	.270	2.360
Between Years of Foliage Within Tree Ages	.849	1.213	2.076*

*Indicates F ratio significant @ $p \leq .05$; F ratios in parentheses are those reported in the TIR.

[‡] The probability level for this F ratio is .0591.

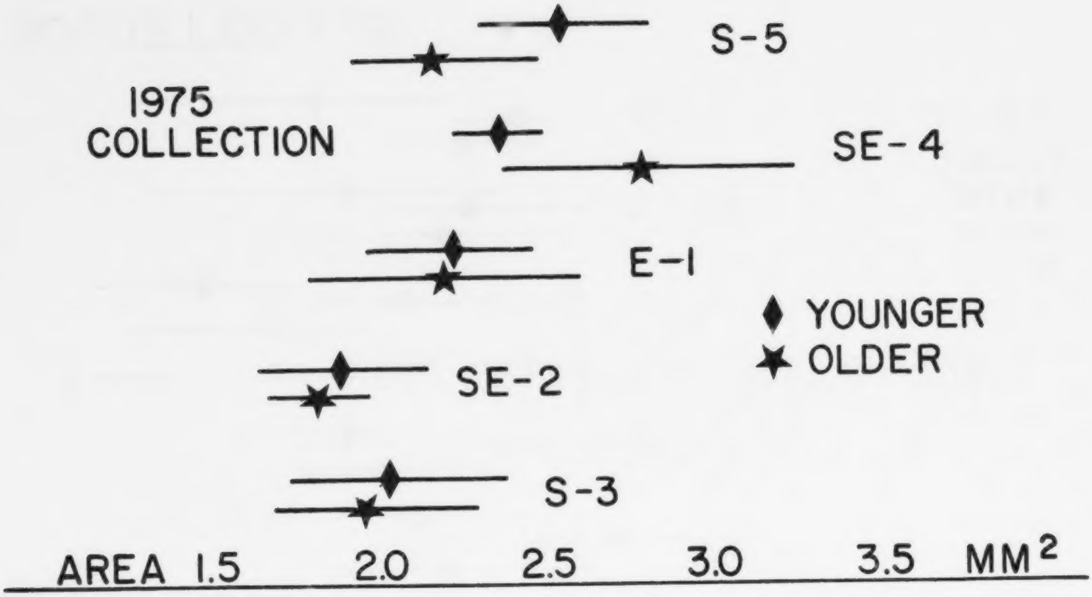


Figure 5.44. Mean values and 95% confidence intervals for fascicular cross-sectional area, upper crown, younger and older trees.

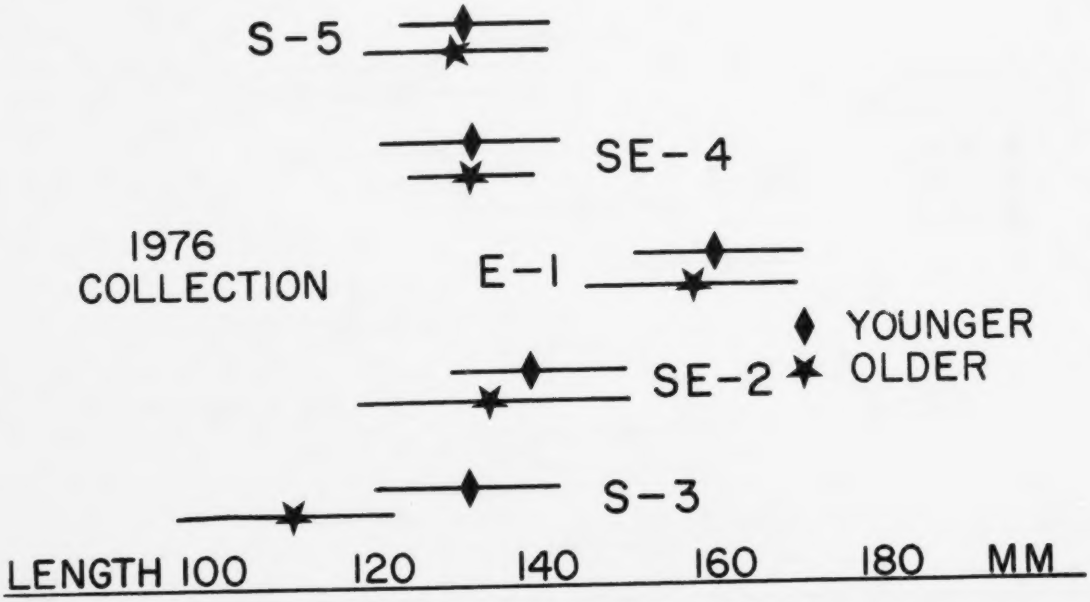


Figure 5.45. Mean values and 95% confidence intervals for needle length, upper crown, younger and older trees.

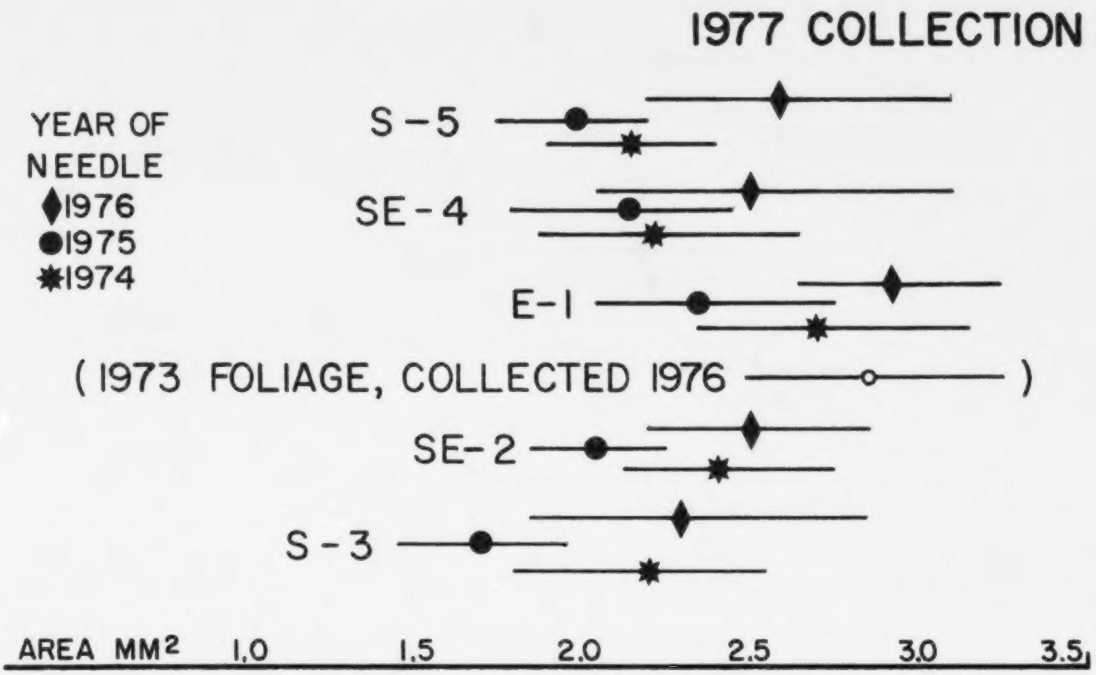


Figure 5.46. Mean values and 95% confidence intervals for fascicular cross-sectional area, all tree ages.

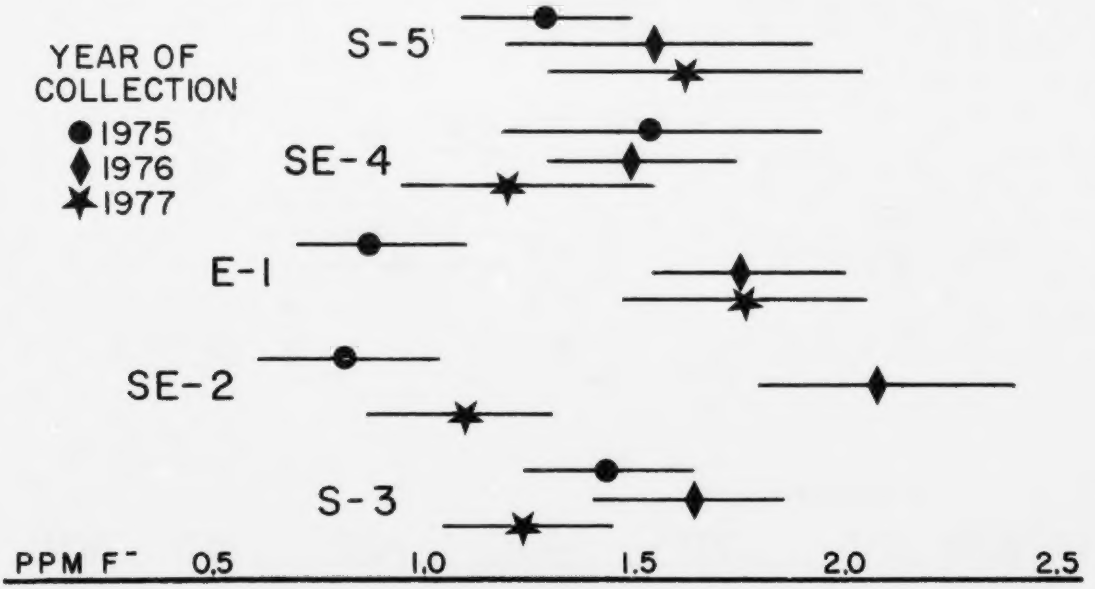


Figure 5.47. Mean values and 95% confidence intervals for fluoride, all tree ages, all years' foliage.

for site E-1.) In the 1976 collection, 15-month foliage (1975 needles) had the smallest cross-sectional area. During 1977, 15-month foliage (1976 needles) generally had the greatest area at each site. This suggests that those biological and edaphic variables which influence fascicular cross-sectional area are similar across all sites.

Significant ($p \leq .05$) treatment effects between sites were noted for ppm fluoride in the 1975 collection, while no treatment effects were noted in the 1976 collection (Table 5.1). The treatment effect between sites in the 1977 collection (Table 5.1) was significant at $p \leq .06$ ($p = .0591$). Figure 5.47 depicts the mean values and 95% confidence intervals for fluoride in the upper crown position of all years' foliage combined for all sites from three years' collections.

The differences in mean values for fluoride between sites from the 1975 collection are obvious (Figure 5.47). The same trend was similar on the 1976 collection. In 1977, differences in fluoride between sites were becoming apparent, with the exception of foliage from E-1 where the mean values were similar to those measured in 1975.

Table 5.2 shows F ratios for the four-level nested anovas for 1975-1976 collections and 1975-1977 collections. The F ratios enclosed in parentheses are those reported in the TIR in which data from the Whitetail site were used.

In both 1975-1976 and 1975-1977 collections, significant treatment effects were noted between sites within collections for all variables and between collections for fluoride only (Table 5.2). These effects were the same whether the Yaeger Butte site or the Whitetail site was used.

These results are consistent and support those reported in the TIR. Samples of ponderosa pine foliage collected from geographically separate sites, from different tree ages within a site, or from different ages of foliage cannot a priori be considered to be samples from the same population for all characteristics.

Anova with GB-1, BNW-1, BNW-2, BNW-3 (1976 and 1977 Collections)

F ratios for three-level nested anova computed for all sites on 1976 and 1977 collections are shown in Table 5.3. Significant treatment effects ($p \leq .05$) between sites for the 1976 collection are shown for fluoride and needle length, and between the years of foliage for fluoride.

The mean values and 95% confidence intervals for needle length collected in 1976 from younger and older trees at all sites are shown in Figure 5.48. Although the F ratio for the treatment between tree ages (Table 5.3) was not significant ($p = .1266$), data on Figure 5.48 are shown between tree ages because of the difference previously shown for site S-3 (Figure 5.45 and Table 5.1). The mean values (Figure 5.48) for needle length of foliage from younger trees at GB-1, S-5, SE-4, SE-2, and S-3 (younger trees) are very similar. The BNW sites showed a difference in mean values for older and younger trees (younger < older), although the differences were not significant. However, the differences between younger and older trees were approximately 10 mm at each of the BNW sites.

TABLE 5.2. F RATIOS FOR FLUORIDE, NEEDLE LENGTH, AND FASCICULAR CROSS-SECTIONAL AREA FOR THE FOUR-LEVEL NESTED ANOVAS FOR THE 1975-1976 AND 1975-1977 COLLECTIONS

Level	Fluoride	Needle Length	Fascicular Cross-Sectional Area
	1975-1976 Collections Variable		
Between Collections	8.957* (7.557)*	.007 (.048)	.350 (1.252)
Between Sites Within Collections	9.598* (5.925)*	6.584* (10.369)*	5.832* (6.289)*
Between Tree Ages Within Sites	.695 (1.731)	1.937 (1.481)	1.827 (1.293)
Between Years of Foliage Within Tree Ages	1.019 (.784)	.958 (1.066)	.708 (.741)
	1975-1977 Collections Variable		
Between Collections	4.622*	.0118	.290
Between Sites Within Collections	7.244*	8.801*	4.239*
Between Tree Ages Within Sites	.829	1.247	1.582
Between Years of Foliage Within Tree Ages	.954	1.050	1.082

*Indicates F ratio significant @ $p \leq .05$; F ratios in parentheses are those reported in the TIR.

TABLE 5.3. F RATIOS FOR FLUORIDE, NEEDLE LENGTH, AND FASCICULAR CROSS-SECTIONAL AREA FOR THE THREE-LEVEL NESTED ANOVAS FOR THE 1976 AND 1977 COLLECTIONS

Level	Fluoride	Needle Length	Fascicular Cross-Sectional Area
	1976 Collection Variable		
Between Sites	315.218*	6.951*	2.516
Between Tree Ages Within Sites	.563	1.693	1.834
Between Years of Foliage Within Tree Ages	1.920*	.978	1.185
	1977 Collection Variable		
Between Sites	99.448*	25.632*	1.777
Between Tree Ages Within Sites	.560	.294	1.482
Between Years of Foliage Within Tree Ages	1.956*	1.382	1.454[1]

*Indicates F ratio significant @ $p \leq .05$.

[1] The probability level for this F ratio is .0554.

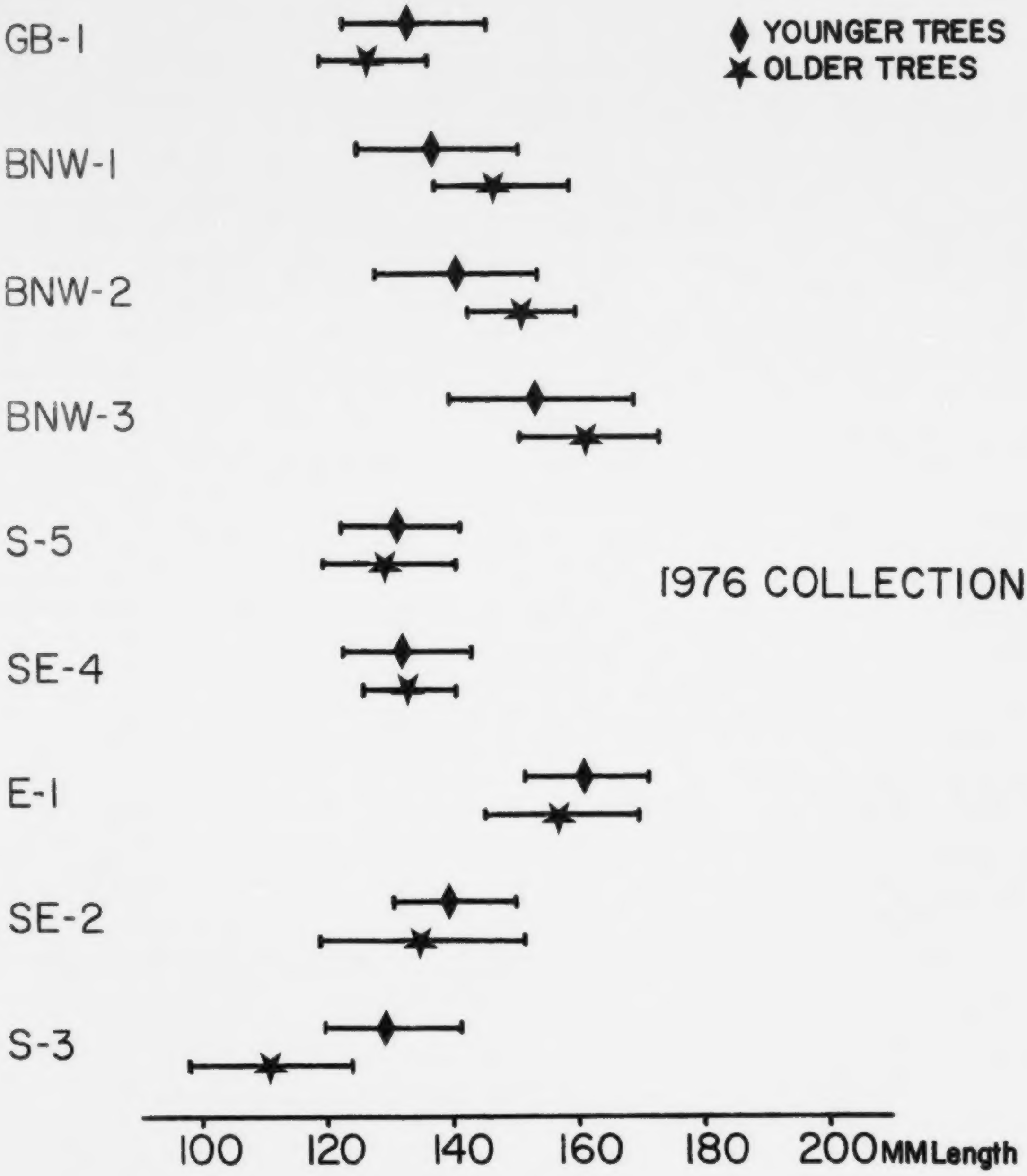


Figure 5.48. Mean values and 95% confidence intervals for needle length, younger and older trees.

The mean values and 95% confidence intervals for needle cross-sectional area collected in 1977 from younger and older trees at all sites are shown in Figure 5.49. This data is shown for tree ages because of the differences evident at S-5 and SE-4 (Figure 5.44 and Table 5.1). Although F ratios for the treatment between tree ages for cross-sectional area were not significant at $p \leq .05$, they were at $p \leq .10$ ($p = .095$). Although all older trees sampled did not have needles with fascicular cross-sectional areas larger than younger trees (and vice versa), the tree ages are different at some sites. Following the trend evident in needle length (Figure 5.48), the GB-1 site did not have needles which were either larger or smaller in cross-sectional area than needles from the other sites.

The mean values and 95% confidence intervals for fluoride in 1973, 1974, and 1975 needles from the 1976 collection at all sites are shown in Figure 5.50. It is obvious that the years of foliage were different (1975 < 1974 or 1973) within GB-1 as well as between GB-1 and all other sites regarding total fluoride. BNW-1 had higher fluoride levels than S-5, SE-4, E-1, SE-2, or S-3. However, only GB-1 showed an obvious difference in fluoride between years of foliage origin.

In the 1977 collection, significant treatment effects ($p \leq .05$) (Table 5.3) were detected between sites for fluoride and needle length, and between years of foliage for fluoride and fascicular cross-sectional area. The mean values and 95% confidence intervals for fascicular cross-sectional area for 1974, 1975, and 1976 foliage from the 1977 collection are shown on Figure 5.51 for GB-1 and the BNW sites. The same data are shown on Figure 5.46 for the other sites. A similar trend is evident in both figures: The 1976 foliage had a larger average cross-sectional area than 1975 foliage; however, 1975 foliage was not significantly different than 1974 foliage.

Figure 5.52 shows the mean values and 95% confidence intervals for fluoride in 1974, 1975, and 1976 foliage collected in 1977 from all sites. The difference in fluoride between years of foliage from GB-1 was clear; 1975 foliage from this site showed approximately the same average fluoride content in 1976 as in 1977 (Figures 5.50 and 5.52, respectively), and values for 1974 foliage were reduced from about 60 ppm (Figure 5.50) to about 43 ppm (Figure 5.52). The mean values for fluoride in foliage from BNW-1 were greater in 1976 than in 1977 (Figures 5.50 and 5.52, respectively). Figure 5.53 shows the mean values and 95% confidence intervals for fluoride in each year's foliage for 1976 and 1977 collections from BNW sites only.

During the 1977 collection, fascicular sheaths from all sites were retained for fluoride and sulfur analyses. Fluoride determinations have been completed, and anova was computed on the data. F ratios for the indicated levels are shown on Table 5.4. Significant treatment effects between sites and between years of foliage were detected. Mean values and 95% confidence intervals for fluoride in fascicular sheaths from each year of foliage for the 1977 collection period are shown in Figure 5.54. Differences in mean fluoride values between sites and between years of foliage are obvious. None of these values exceeded 6 ppm for sites S-5, SE-4, E-1, SE-3, or S-3. However, at BNW-2 and BNW-3, only 1976 sheaths had values below 6 ppm. Mean fluoride values for 1974 and 1975 foliage at these two sites and mean fluoride values for all years of foliage at BNW-1 were greater than 7 ppm.

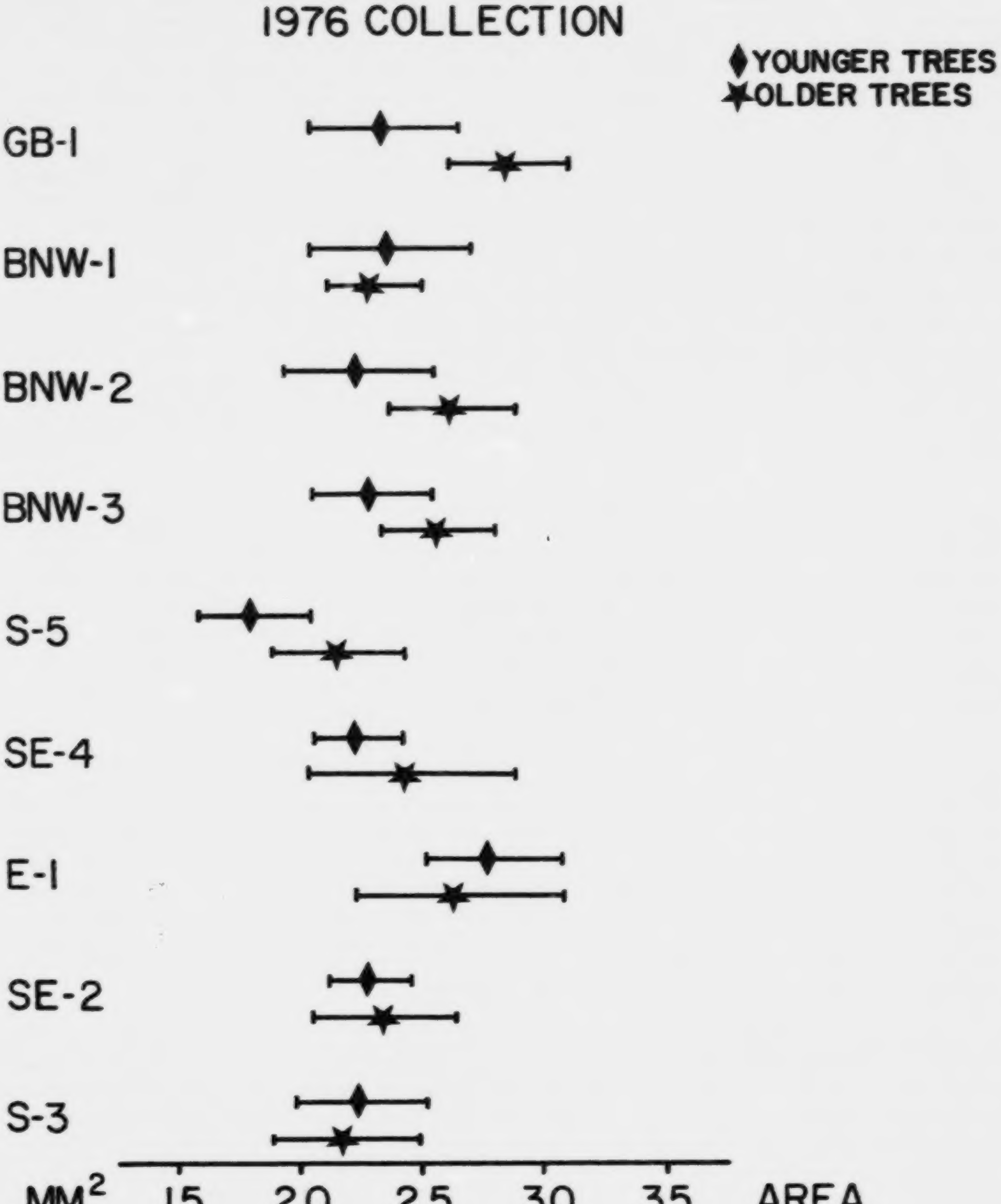


Figure 5.49. Mean values and 95% confidence intervals for needle cross-sectional area, younger and older trees.

The mean values and 95% confidence intervals for needle cross-sectional area collected in 1977 from younger and older trees at all sites are shown in Figure 5.49. This data is shown for tree ages because of the differences evident at S-5 and SE-4 (Figure 5.44 and Table 5.1). Although F ratios for the treatment between tree ages for cross-sectional area were not significant at $p \leq .05$, they were at $p \leq .10$ ($p = .095$). Although all older trees sampled did not have needles with fascicular cross-sectional areas larger than younger trees (and vice versa), the tree ages are different at some sites. Following the trend evident in needle length (Figure 5.48), the GB-1 site did not have needles which were either larger or smaller in cross-sectional area than needles from the other sites.

The mean values and 95% confidence intervals for fluoride in 1973, 1974, and 1975 needles from the 1976 collection at all sites are shown in Figure 5.50. It is obvious that the years of foliage were different (1975 < 1974 or 1973) within GB-1 as well as between GB-1 and all other sites regarding total fluoride. BNW-1 had higher fluoride levels than S-5, SE-4, E-1, SE-2, or S-3. However, only GB-1 showed an obvious difference in fluoride between years of foliage origin.

In the 1977 collection, significant treatment effects ($p \leq .05$) (Table 5.3) were detected between sites for fluoride and needle length, and between years of foliage for fluoride and fascicular cross-sectional area. The mean values and 95% confidence intervals for fascicular cross-sectional area for 1974, 1975, and 1976 foliage from the 1977 collection are shown on Figure 5.51 for GB-1 and the BNW sites. The same data are shown on Figure 5.46 for the other sites. A similar trend is evident in both figures: The 1976 foliage had a larger average cross-sectional area than 1975 foliage; however, 1975 foliage was not significantly different than 1974 foliage.

Figure 5.52 shows the mean values and 95% confidence intervals for fluoride in 1974, 1975, and 1976 foliage collected in 1977 from all sites. The difference in fluoride between years of foliage from GB-1 was clear; 1975 foliage from this site showed approximately the same average fluoride content in 1976 as in 1977 (Figures 5.50 and 5.52, respectively), and values for 1974 foliage were reduced from about 60 ppm (Figure 5.50) to about 43 ppm (Figure 5.52). The mean values for fluoride in foliage from BNW-1 were greater in 1976 than in 1977 (Figures 5.50 and 5.52, respectively). Figure 5.53 shows the mean values and 95% confidence intervals for fluoride in each year's foliage for 1976 and 1977 collections from BNW sites only.

During the 1977 collection, fascicular sheaths from all sites were retained for fluoride and sulfur analyses. Fluoride determinations have been completed, and anova was computed on the data. F ratios for the indicated levels are shown on Table 5.4. Significant treatment effects between sites and between years of foliage were detected. Mean values and 95% confidence intervals for fluoride in fascicular sheaths from each year of foliage for the 1977 collection period are shown in Figure 5.54. Differences in mean fluoride values between sites and between years of foliage are obvious. None of these values exceeded 6 ppm for sites S-5, SE-4, E-1, SE-3, or S-3. However, at BNW-2 and BNW-3, only 1976 sheaths had values below 6 ppm. Mean fluoride values for 1974 and 1975 foliage at these two sites and mean fluoride values for all years of foliage at BNW-1 were greater than 7 ppm.

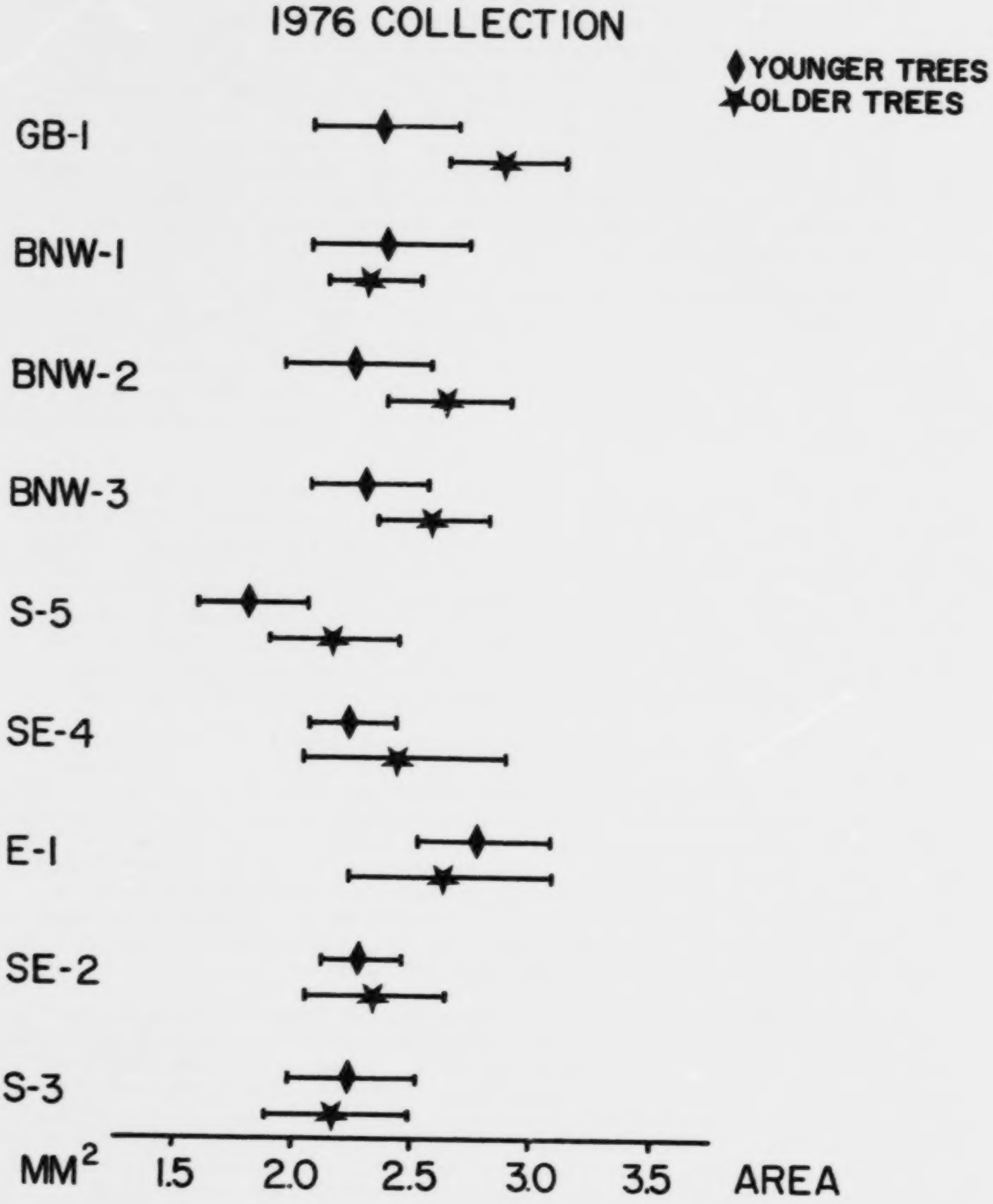


Figure 5.49. Mean values and 95% confidence intervals for needle cross-sectional area, younger and older trees.

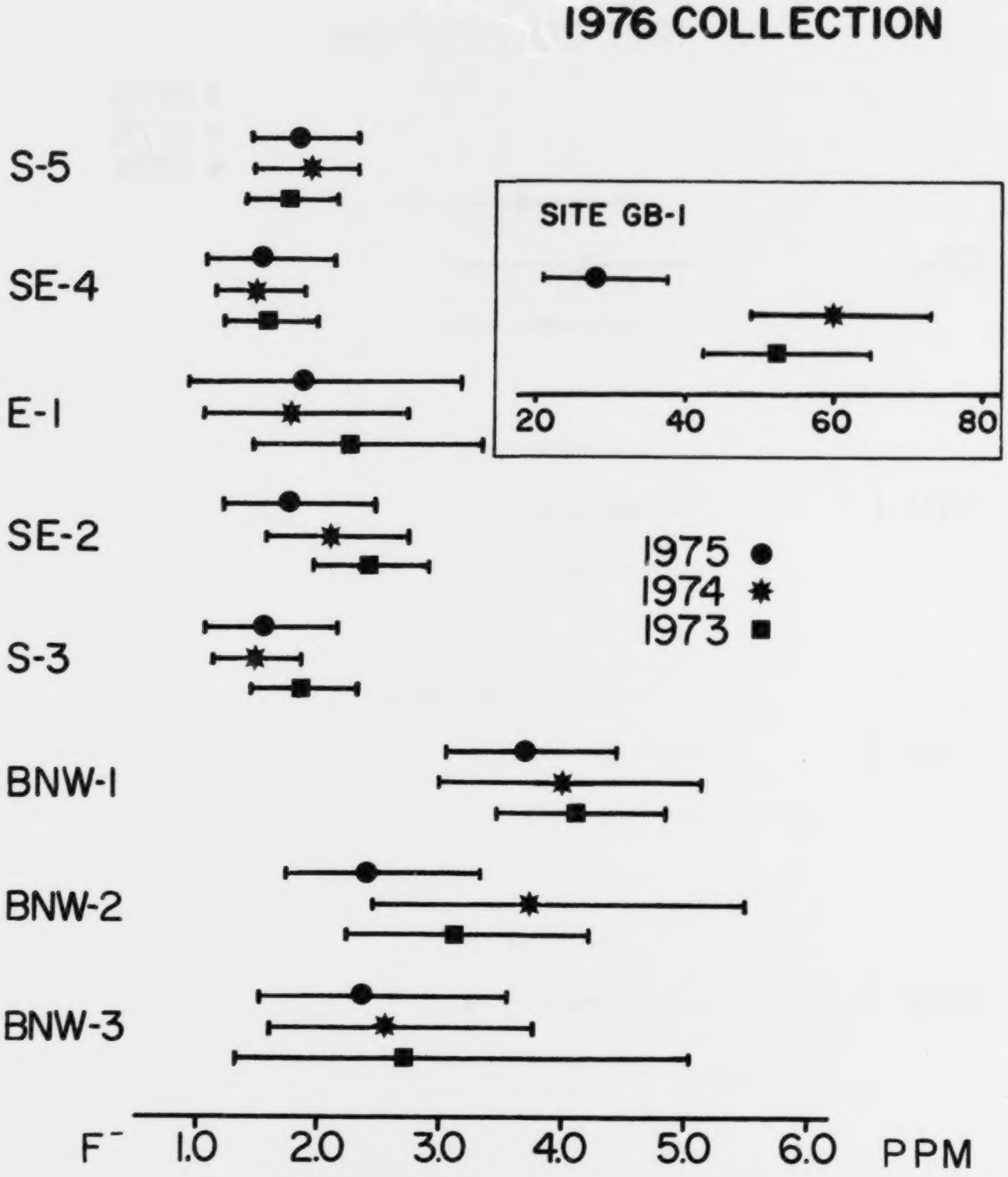


Figure 5.50. Mean values and 95% confidence intervals for fluoride in 1973, 1974, and 1975 foliage.

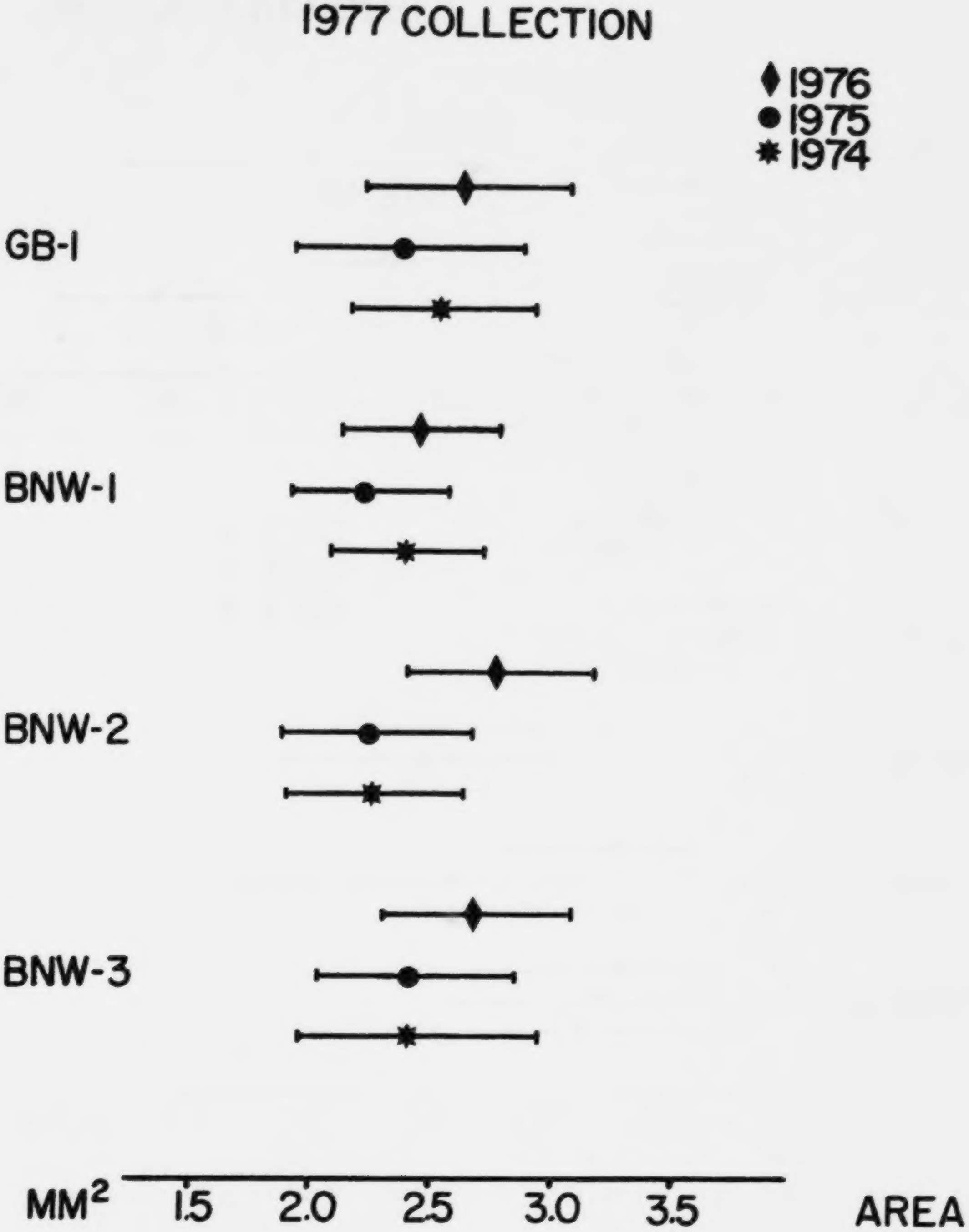


Figure 5.51. Mean values and 95% confidence intervals for fascicular cross-sectional area in 1974, 1975, and 1976 foliage.

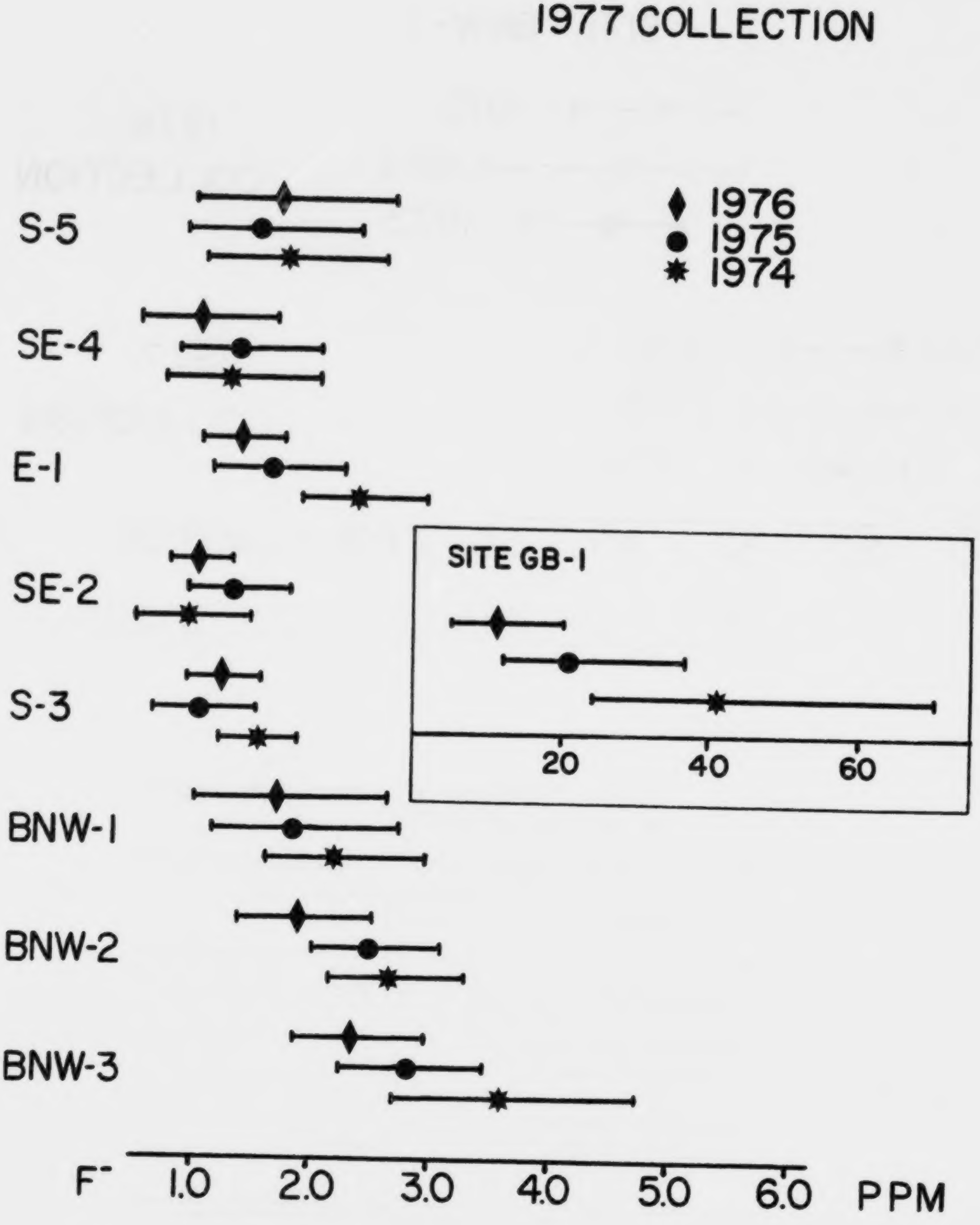


Figure 5.52. Mean values and 95% confidence intervals for fluoride in 1974, 1975, and 1976 foliage.

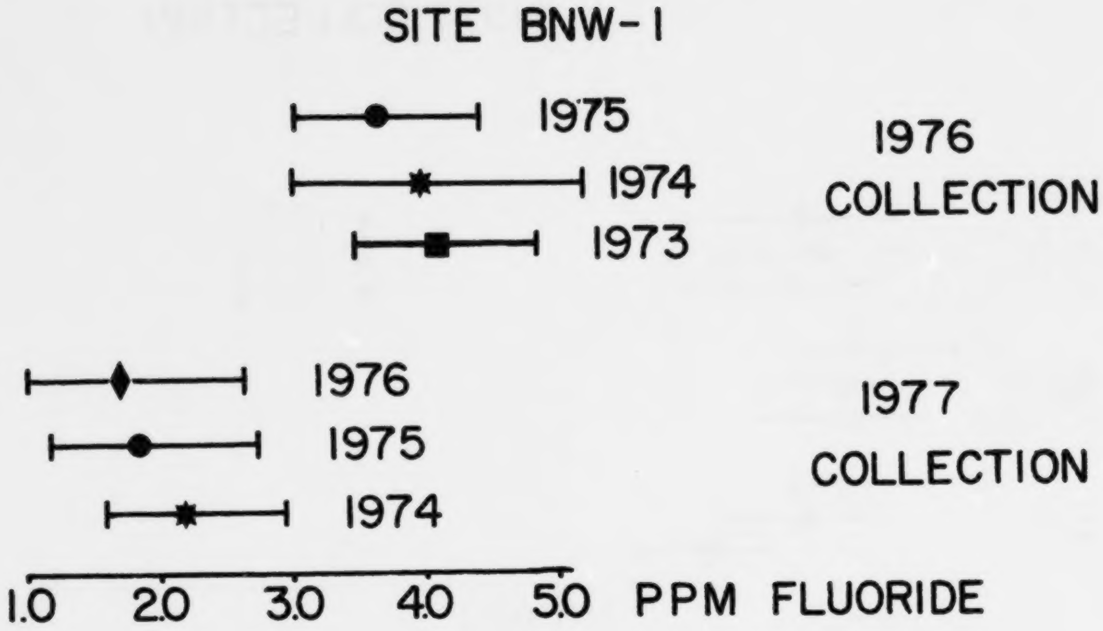


Figure 5.53. Mean values and 95% confidence intervals for fluoride in 1973, 1974, 1975, and 1976 foliage at site BNW-1.

TABLE 5.4. F RATIOS FOR FLUORIDE IN FASCICULAR SHEATHS FOR THE 1977 COLLECTION

Level	Variable Fluoride in Sheaths
Between Sites	185.532*
Between Tree Ages Within Sites	.133
Between Years of Foliage Within Tree Ages	6.886*

*Indicates F ratio significant @ $p \leq .05$.

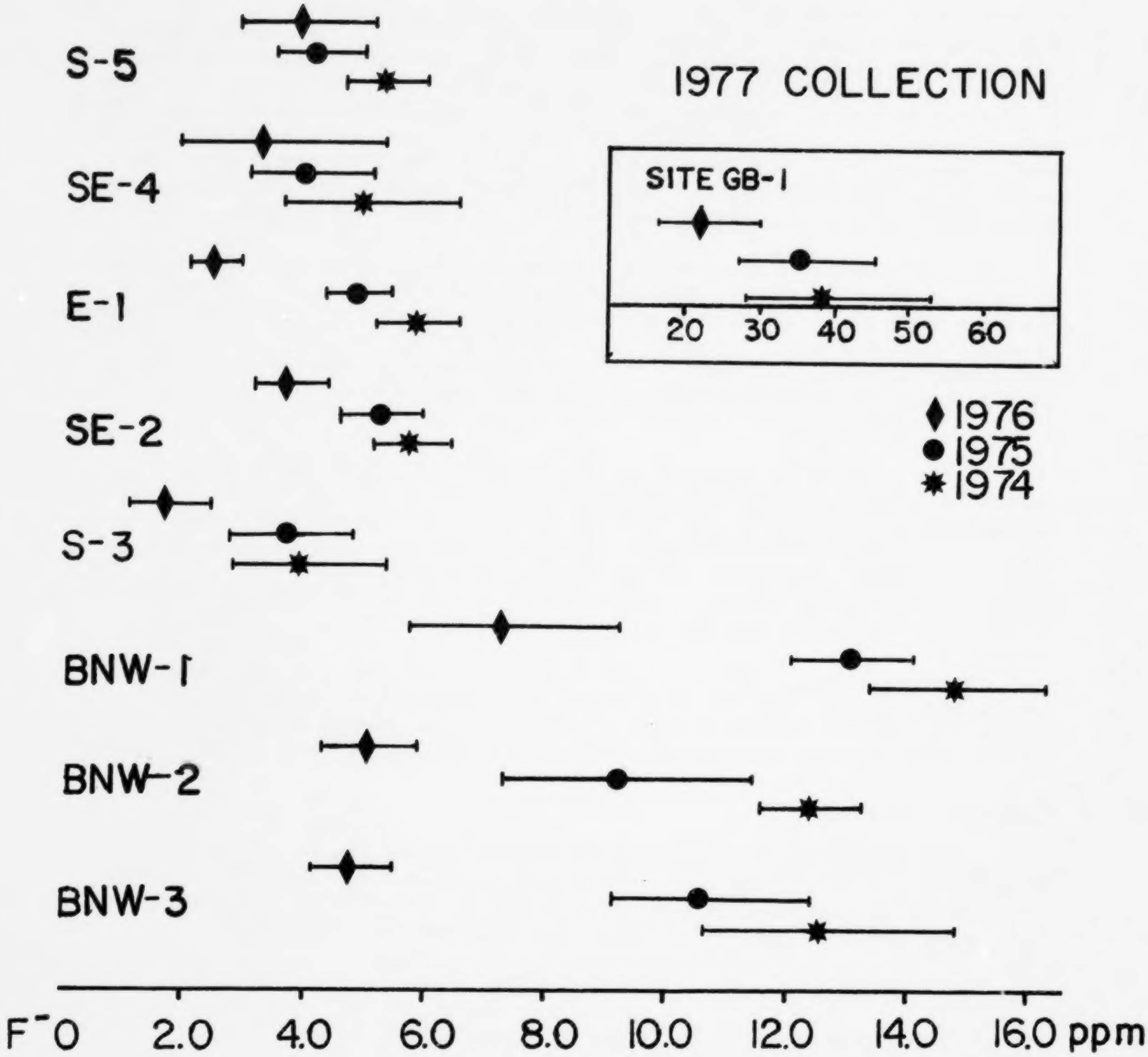


Figure 5.54. Mean values and 95% confidence intervals for fluoride in fascicular sheaths of 1974, 1975, and 1976 foliage.

The mean values and 95% confidence intervals for sulfur in pine needles from S-3 for 1976 and 1977 collections are shown in Figure 5.55. Figure 5.56 shows results of the 1977 collection from the BNW sites. As can be seen, foliage from BNW-2 generally contained less sulfur than foliage from BNW-1 or BNW-3. Figures 5.57 and 5.58 show mean values and 95% confidence intervals for sulfur in foliage from the 1976 and 1977 collections from BNW-1 and BNW-3. BNW-1 showed a slight reduction in mean sulfur concentrations in 1977 below those measured in 1976 (Figure 5.57), as did site S-3 (Figure 5.55). The sulfur values at BNW-3 did not show a parallel decrease for the 1977 collection; rather there is a slight increase in 1977 over 1976 (Figure 5.58).

Figure 5.59 shows mean values and 95% confidence intervals for sulfur in fascicular sheaths from the three BNW sites collected in 1977. As with fluoride (Figure 5.54), the mean sulfur concentration increased with increasing year of foliage origin. Figures 5.60 and 5.61 show the mean values and 95% confidence intervals for sulfur in fascicular sheaths from BNW-1 and BNW-3, respectively, for 1976 and 1977 collections. Sulfur decreased in 1977 from the 1976 levels at BNW-1 (Figure 5.60) but not at BNW-3 (Figure 5.61).

Figure 5.62 shows the mean values and 95% confidence intervals for sulfur in needles and fascicular sheaths from GB-1, 1977 collection. Sulfur in needles increased from the 1977 to the 1976 years of foliage, remained very similar through 1974 foliage, and decreased from 1974 through 1972. In contrast, sulfur concentrations in fascicular sheaths showed an increase with older foliage. Figure 5.63 shows the mean values and 95% confidence intervals at GB-1 for sulfur in ponderosa pine needles, 1976 and 1977 collections.

The mean values shown on Figure 5.62 are about twice those measured at the Colstrip sites (compare with Figure 5.55 and 5.56). While there was a slight decrease in sulfur at GB-1 in 1977 from the levels measured in 1976, the decrease was not observed in all years of foliage (Figure 5.63).

Understory Species

Basic statistics for fluoride in understory species collected at sites S-5, SE-4, E-1, SE-2, and S-3 are arrayed in Table 5.5. Fluoride analyses have yet to be completed on the BNW and GB-1 sites. Sulfur measurements on understory species from any of the sites have not been completed.

DISCUSSION

Growth/Health/Damage Characteristics of Pine Foliage

Quantification of the growth/health/damage characteristics of pine foliage exposed to pristine and chronically-polluted environments for 15 to 51 months has revealed very subtle pathological damage in ponderosa pine foliage in both environments. This damage may not be apparent to even trained and experienced air pollution field researchers. For instance, the upper and lower canopies of the ponderosa pine trees at the Billings GB-1 site are not so visibly damaged that one can use field observations to conclude that pines here are retaining less healthy or even less needles than those at the pristine S-3 site.

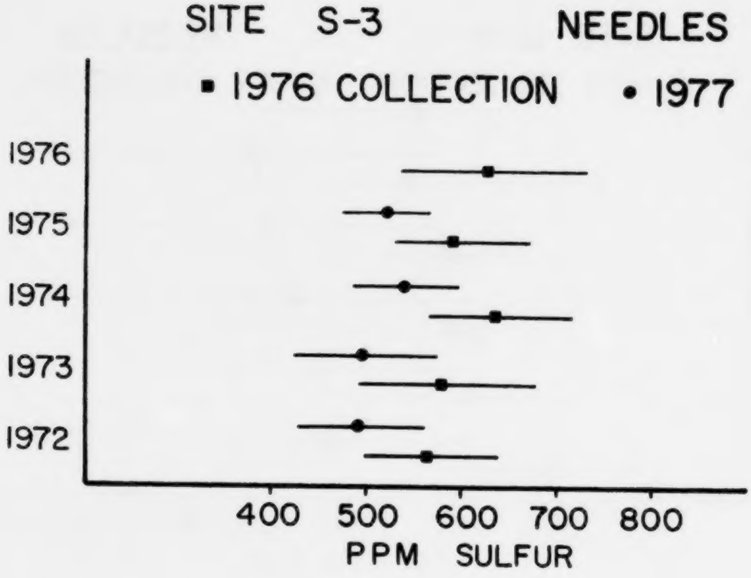


Figure 5.55. Mean values and 95% confidence intervals for sulfur in pine needles from site S-3.

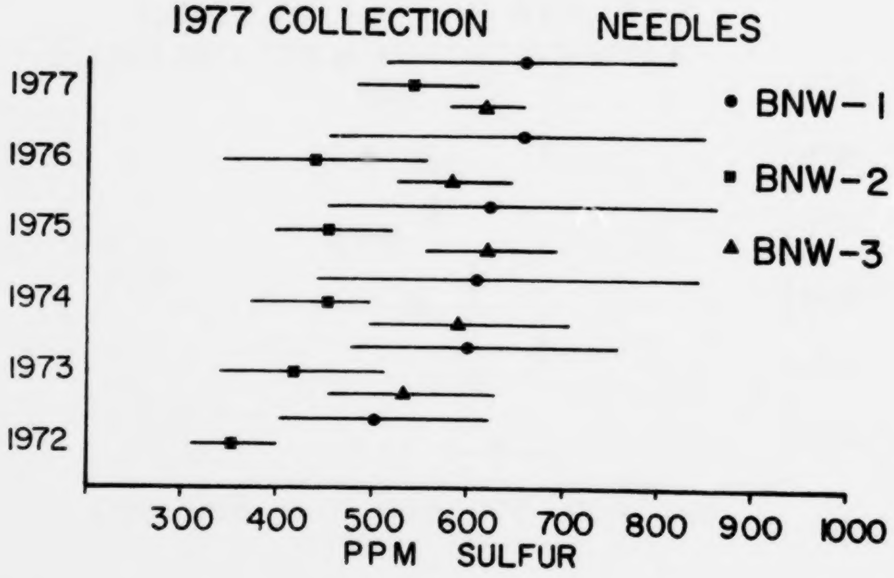


Figure 5.56. Mean values and 95% confidence intervals for sulfur in pine needles from the BNW sites.

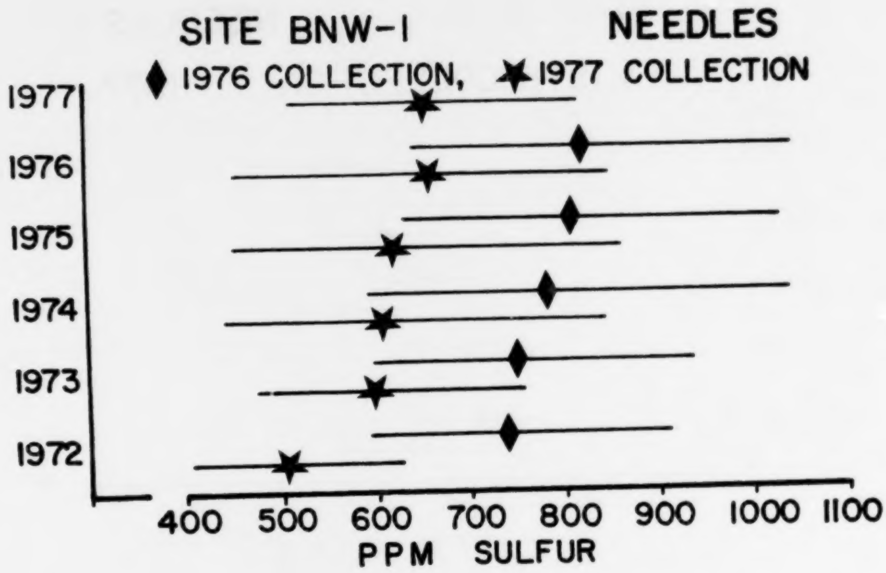


Figure 5.57. Mean values and 95% confidence intervals for sulfur in pine needles from site BNW-1.

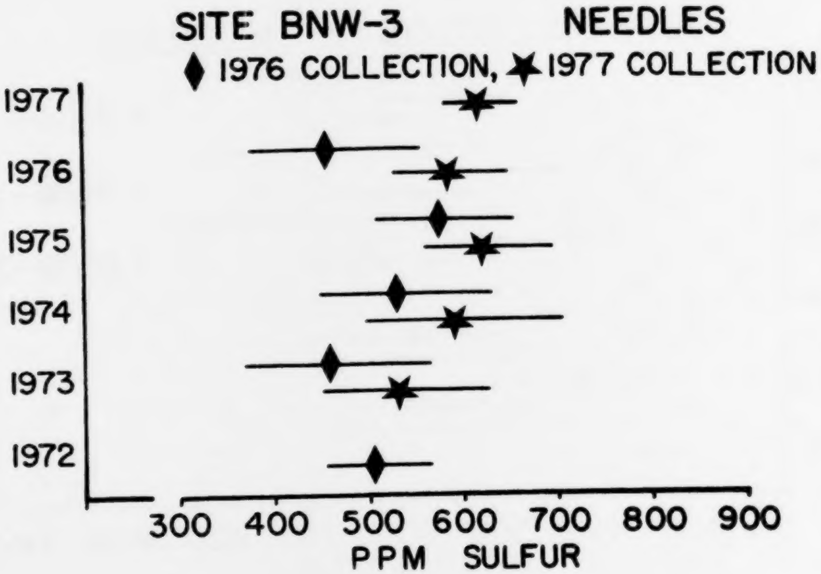


Figure 5.58. Mean values and 95% confidence intervals for sulfur in pine needles from site BNW-3.

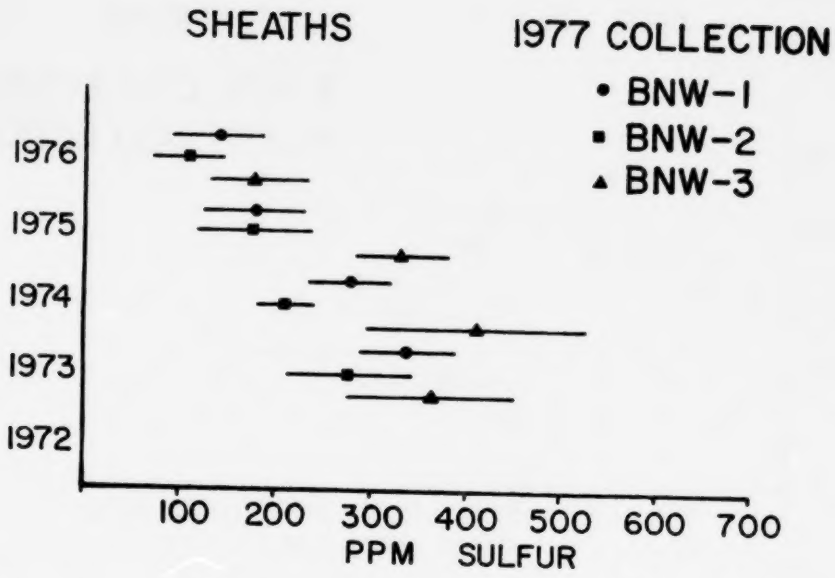


Figure 5.59. Mean values and 95% confidence intervals for sulfur in fascicular sheaths from the BNW sites.

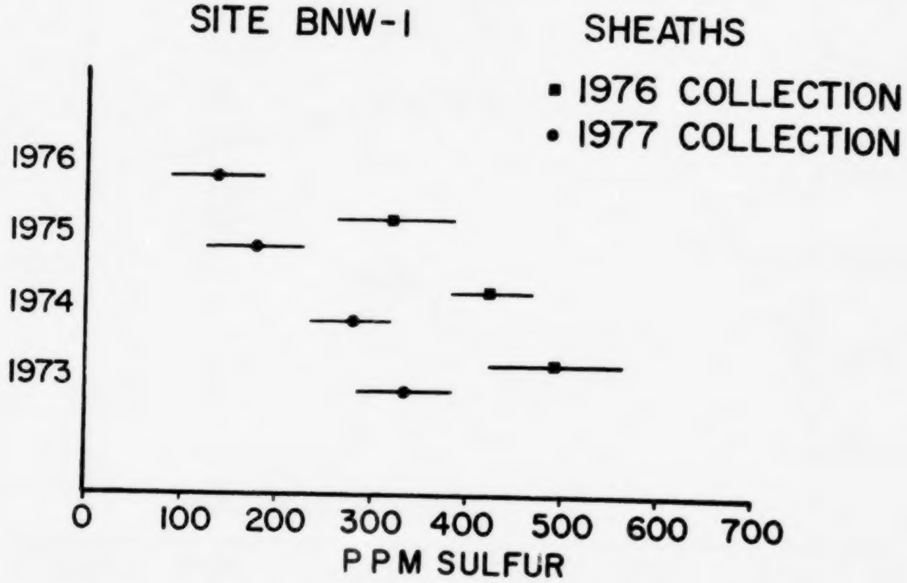


Figure 5.60. Mean values and 95% confidence intervals for sulfur in fascicular sheaths from site BNW-1.

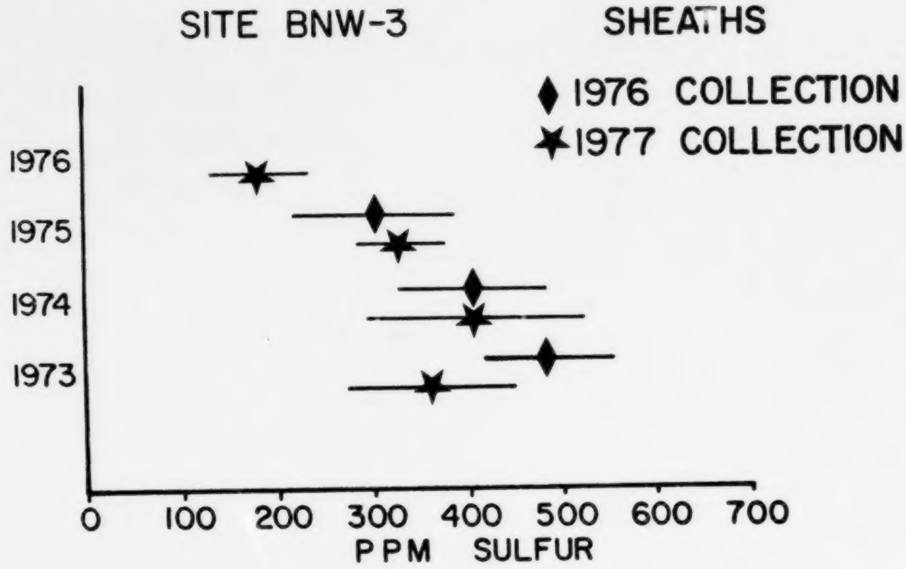


Figure 5.61. Mean values and 95% confidence intervals for sulfur in fascicular sheaths from site BNW-3.

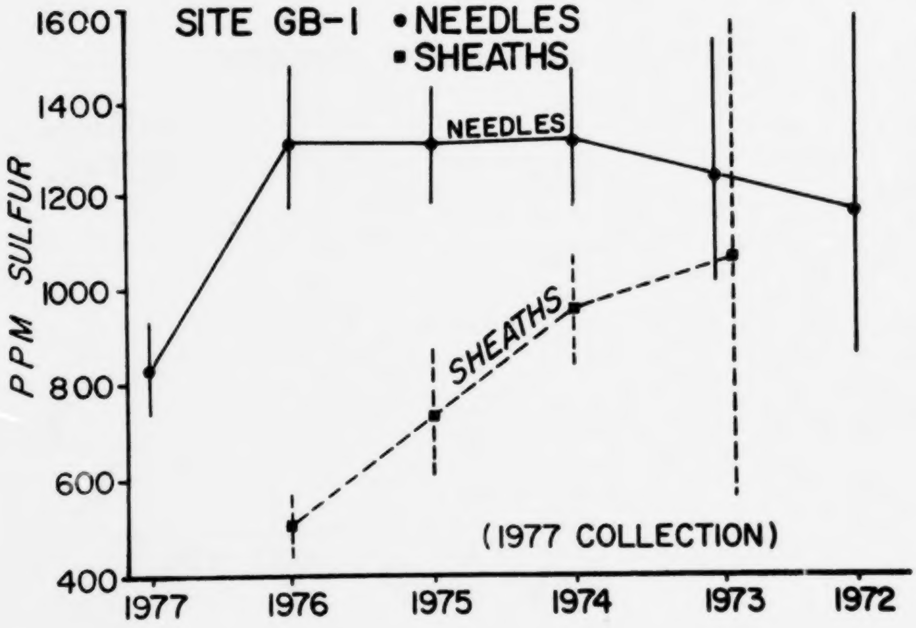


Figure 5.62. Mean values and 95% confidence intervals for sulfur in needles and fascicular sheaths from GB-1.

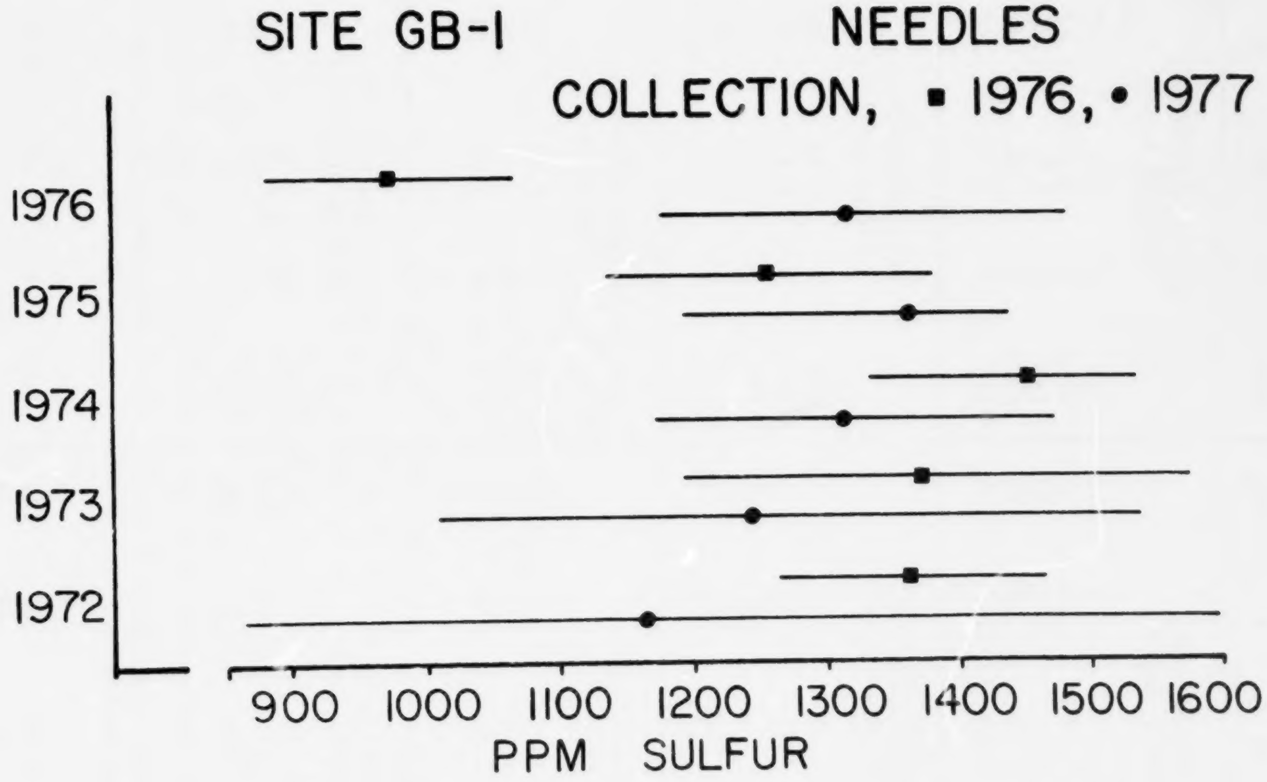


Figure 5.63. Mean values and 95% confidence intervals for sulfur in needles from GB-1.

TABLE 5.5. BASIC STATISTICS FOR FLUORIDE IN UNDERSTORY SPECIES

Site	n	$\overline{x}$	s	$s_{\overline{x}}$	Site	n	$\overline{x}$	s	$s_{\overline{x}}$
Artemesia cana (Silver Sage)					*Artemesia frigida* (Fringed Sage)				
S-5	8	1.9	.29	.10	S-5	8	3.4	.99	.35
SE-4	8	2.71	1.53	.54	SE-4	7	3.8	1.87	.71
E-1	8	2.23	.62	.22	E-1	8	2.8	1.06	.37
SE-2	8	1.88	.72	.25	SE-2	8	1.96	.55	.19
S-3	8	1.28	.43	.15	S-3	8	3.35	.25	.09
Agropyron spicatum (Bluebunch Wheatgrass)					*Rhus trilobata* (Skunkbush)				
S-5	8	.99	.32	.11	S-5	8	1.18	.52	.18
SE-4	8	2.98	1.12	.4	SE-4	8	4.1	1.37	.48
E-1	8	.9	.57	.2	E-1	8	1.94	.51	.18
SE-2	8	1.1	.32	.11	SE-2	8	1.78	.42	.15
S-3	8	2.29	.59	.21	S-3	8	1.96	.62	.22
Yucca glauca (Yucca)					*Festuca idahoensis* (Idaho Fescue)				
S-5	8	1.15	.36	.13	S-5	8	2.28	.49	.17
SE-4	8	2.86	.56	.2	SE-4	8	1.2	1.19	.42
E-1	8	.91	.24	.08	S-3	8	2.03	.92	.32
S-3	8	3.3	.42	.15					
Psoralea sp. (Scurf Pea)					*Andropogon scoparius* (Little Bluestem)				
SE-4	7	4.87	1.46	.55	E-1	8	3.36	1.45	.51
E-1	5	3.4	1.77	.79	SE-2	8	.81	.36	.13
S-3	8	3.36	1.0	.36	S-3	8	.8	.35	.14
Gutierrezia sarothrae (Brown Snakeweed)					*Artemesia ludoniciana* (Prairie Sage)				
S-5	8	3.7	1.03	.36	S-5	6	5.7	4.37	1.78
SE-4	8	3.84	1.00	.35	SE-4	8	5.25	2.28	.81
SE-2	8	2.2	.69	.24					

(continued)

TABLE 5.5. BASIC STATISTICS FOR FLUORIDE IN UNDERSTORY SPECIES (continued)

Site	n	$\bar{x}$	s	$s_{\bar{x}}$	Site	n	$\bar{x}$	s	$s_{\bar{x}}$
Balsamorhiza sagittata (Arrowleaf Balsamroot)					*Lupinus sp.* (Lupine)				
S-5	8	4.14	1.52	.54	S-5	8	2.44	.35	.12
SE-4	8	4.58	1.48	.52	S-3	8	3.44	.72	.26
Artemesia tridentata (Big Sage)					*Stipa comata* (Needle and Thread)				
SE-2	8	.84	.54	.19	SE-2	8	1.89	.63	.22
Juniperus scopulorum (Rocky Mountain Juniper)					*Prunus virginiana* (Chokecherry)				
SE-2	8	2.24	.42	.15	S-3	8	.75	.19	.07

Our studies during the last three years have taught us that chronic air pollution damage is not necessarily manifested by pine foliage exposed for 12 or even 24 months. Damage must be measured during the entire tenure of foliage on the trees. Thus, if field researchers find only slight pathological symptoms on current to three-year-old foliage (27 months), they do not necessarily have scientific evidence significant enough to conclude that chronic air pollution damage is not occurring. In fact, after this study in southeastern Montana is completed, it might be advantageous to do some redefining and to separate the rapid chronic air pollution damage (one growing season) occurring in the San Bernardino Forest of California (Miller *et al.*, 1977) and the Flathead National Forest of Montana (Carlson, 1978) from the slow, insidious chronic damage (two to five growing seasons) we are finding at a few of the Colstrip sites and at the Billings GB-1 site. It is very likely that past air pollution field investigations were utilizing chronically-damaged coniferous forest sites as controls to compare or quantify impacts of the acute and/or severe chronic damage to conifer sites they were studying. If one compares a low chronically-damaged conifer site to a severe chronically-damaged conifer site, one really has not accurately ascertained the full impacts of the air pollution episodes.

Earlier in the results section, we cautioned the reader on the use of a single-year study to determine air pollution impacts on the growth/health/damage characteristics of pine foliage. We hope that our reasons for suggesting this caution become apparent as one compares the results of our 1977 findings, presented in Figures 5.3 through 5.24, to data presented on the 1973 and 1974 foliage exposed for three years (Figures 5.25 through 5.33) or for two years (Figures 5.34 through 5.43).

Several of the growth/health/damage characteristics quantified during a given study period tend to show the same patterns as other characteristics which occur over a longer period of time. The first such characteristic is needle retention, which is illustrated in Figures 5.3, 5.25, and 5.34. One notes that the trends on all of these graphs are identical. All three, particularly Figure 5.3, demonstrate that needles are continuously being cast from all-aged internodes, especially between the 39- and 51-month exposure periods at the Colstrip sites. At the chronically-polluted GB-1 site, the major amount of premature needle casting occurred between the 27- and 39-month exposure period. During the 1978 study season, we will continue to quantify percent needle retention on older foliage (1973 and 1974) where possible at several of the pine sites.

Percent healthy needles is a characteristic which also maintains a uniform trend when quantified over several growing seasons (Figure 5.4). The most obvious changes in the number of healthy needles were quantified at GB-1, and the largest amount of premature needle casting occurred at this Billings site.

The amount of needle mottle quantified during a single study period is not necessarily a characteristic which an investigator can utilize to determine air pollution impacts. For instance, one notes in Figure 5.5 that the prevalence of this pathology was less in 15-month-old foliage from the GB-1 site than on foliage from the three Colstrip sites; mottle was also less prevalent on 51-month exposed foliage from GB-1 than on foliage from six Colstrip sites. Even in quantifications of the amount of needle tissue damaged by mottling (Figure 5.13), the two Colstrip sites had similar damage on 15-, 27-, and 51-month-old foliage. The results we have thus far accumulated strongly suggest that premature casting from various-aged internodes is removing substantial amounts of mottled needles.

We believe that needle tip necrosis (or tip burn) can be used in both single and extended study periods. However, one should use some caution when studying this pathology on older foliage. As depicted in Figure 5.36, the prevalence of tip burn remained very similar on 27- and 51-month-old foliage from BNW-3 and GB-1 during this extended period. An investigator quantifying this pathology on various-aged foliage might infer that it ceases after needles reach a certain age. If this were true, all the prematurely cast needles of this-aged foliage (Figures 5.3 and 5.34) would not show any tip burn. This is extremely improbable because, as the data on Figures 5.6, 5.14, 5.27, and 5.36 indicate, the incidence of tip necrosis keeps pace with premature needle casting in chronically-polluted areas.

We have placed quotation marks around the word "pristine" when describing the remote pine sites in southeastern Montana (S-5, SE-4, and S-3), because basal scale and basal necrosis were prevalent and quantified throughout the area before the Colstrip power plants began operations.

Event rainwater samples collected before and after the Colstrip coal-fired units went on-line had pH values ranging from over 8.0 to lows of 4.1 (CO_2 free). Because of these lower acidic levels, it is our belief that the Colstrip study area was not pristine before the power plants began operations, even though no detectable levels of phytotoxic gases, except for ozone (.03 ± .01 ppm), were reported after thousands of hours of ambient air monitoring by EPA (Northern Cheyenne Tribe, 1976) and the Montana Department of Health and Environmental Sciences (1976). Thus, while we have continued to utilize the term "pristine" to describe several of our ponderosa pine-skunkbush sites located 25 to 80 km from Colstrip, the term relates only to the lack of phytotoxic gases in the ambient air and not to event rainfall with pH values below 5.4. Data from our precipitation chemistry studies of event rainfalls in southeastern Montana were reported by Gordon *et al.* (1978b) in an annual report to ERDA (former name of present DOE) which is currently in draft form.

After nine years of histological studies, we now consider basal scale and basal necrosis to be different manifestations of the same pathology. This long period of indecision is too complex to be discussed here, but it should be noted that other investigators, including Wood and Pennypacker (1975), Farrier (1972), and Keifer and Saunders (1972), have reported that basal needle necrosis can be either identified insects, mites, or unidentified entomological species. We do not doubt that insects are present beneath the fascicular sheaths of pine needles and that some of these species feed upon the tissues of the needle base; we have many photographs and photomicrographs of this phenomenon. However, both basal necrosis and basal scale that we have quantified in polluted and pristine areas and checked with histological studies were caused almost invariably by an abiotic agent. This agent is derived from either acidic precipitation or particulate matter which becomes lodged between the fascicular sheath and the needle epidermis, and precipitates off an acidic solution when moistened by rain, dew, or mist.

Figures 5.7 and 5.8 depict the incidence of basal scale and necrosis on pine foliage collected during 1977. The data on both graphs demonstrate that the incidence of basal needle tissue damage increased constantly with the exposure period of the foliage at all nine sites, the exceptions being basal necrosis at BNW-3, S-5, and SE-4 (Figure 5.8). While there may have been significant differences between the incidence of basal scale and basal necrosis on the same-aged foliage from the nine different sites during 1977, this has not been tested and will be reported later in either the EPA Fifth Interim Report or the next DOE Annual Report.

The basal needle pathologies are particularly interesting because they were only slightly more prevalent on the 27- and 39-month old foliage from GB-1 than on foliage from the Colstrip sites. On Figures 5.17 and 5.20, the incidence of both basal scale and basal necrosis demonstrates that these two pathologies were more prevalent on 51-month-old foliage than the majority of other needle pathologies being quantified. Figure 5.30 shows that both 1973 and 1974 needles prematurely cast from the Colstrip site S-5 between the 1976 and 1977 collection periods included a substantial number of needles with necrotic basal tissue. A similar pattern of needles damaged by basal scale and basal necrosis and prematurely cast from the older internodes can be seen in Figures 5.38 and 5.39 at a few of the sites.

Unfortunately, the incidence or even presence of basal needle tissue damage has been and is being ignored by air pollution field researchers. But personnel of the University of Montana Environmental Studies Laboratory have demonstrated that this pathology is present on all conifer species used as bioindicators in various areas of the United States and Canada.

The incidence of pine needle defoliators was fairly constant on all-aged foliage collected within a given site. To us this means that insects causing the damage are endemic and at most sites consistute a very small percentage of the needle pathologies, as attested by the data on Figures 5.17 through 5.20.

Damage caused by the pine needle weevil (*Scythropus elegans*) was also approximately equal on any given year's foliage collected within a site. One of the more interesting aspects of the data thus far gathered is the low incidence of damage caused by this insect at the chronically-polluted GB-1 and BNW-3 sites. Whether this was due to the presence of pollution or was just a coincidence is not known. However, studies over extended periods will probably disclose whether GB-1 ponderosa pine trees simply have escaped a higher endemic population than the Colstrip sites or if air pollution reduced the population.

"Other pathologies" is an inclusive category for damgage caused by unidentified fungi and possible insect punctures (small necrotic spots) and physical damage caused by bruising or strong winds and/or hail. This is an important category which should be utilized and quantified in air pollution studies. It should be noted that very little fungal-caused needle damage has been observed at any of our ponderosa pine sites during the entire study period, and the injury discussed here was caused primarily by unidentified insects, abiotic agents, and physical damage. Figure 5.11 demonstrates that other pathology damage continually increased over exposure periods; it never decreased on 27- to 51-month-old foliage. Because the amount of damage caused by weevil, defoliator, or abiotic pathologies such as basal scale, basal necrosis, mottle, etc. was reduced at some sites during the 39- to 51-month exposure periods by premature needle casting, we suspect that a large portion of the unidentified needle damage quantified in the other pathology category was caused by abiotic factors not related to air pollution.

The other pathology category was not the most prevalent pathology at S-3 (Figure 5.17), although it was responsible for the largest amount of necrosis and constituted the major amount of needle surface damage on 51-month-old foliage (Figure 5.21). This pathology was also very prevalent at sites BNW-3 and GB-1 (Figures 5.19 and 5.20) where only tip necrosis caused more damage to 51-month-old foliage (Figures 5.23 and 5.24). If air pollution field investigations do not utilize a category for needle damage with no readily ascertainable cause, this type of damage may be categorized as air pollution damage.

The measurement of total necrosis is, as previously mentioned, an estimation by the investigators of the total amount of needle surface area of different-aged foliage damaged by all abiotic and biotic causal agents. We also believe that when 40% to 50% of a needle surface becomes necrotic/chlorotic in either pristine or polluted areas, the needle will be cast from the stem within a 12-month period, regardless of its age. It should not be inferred that needles will not be cast until this much of their surface area is damaged; in some areas, pine needles manifesting substantial amounts of basal needle pathologies (basal scale and basal necrosis) can be cast before their leaf surface is substantially damaged.

Figure 5.43 depicts total necrosis on 1973 and 1974 foliage as quantified during the 1976 and 1977 study period. Figure 5.34 illustrates the prevalence of needle retention during these same study periods. When comparing total necrosis to the needle loss in 1973 and 1974 GB-1 foliage during this two-year study period, the prevalence of total necrosis remained constant, while the incidence of needle casting rapidly increased. During this same time, the prevalence of tip burn (Figure 5.36) remained constant at GB-1, and mottling decreased on both years' foliage (Figure 5.37). The incidence of weevil, defoliator, and other pathologies was fairly constant on 1973 and 1974 foliage from GB-1 during these two years.

There are only two alternate explanations for the fact that the prevalence of total necrosis remained constant on 1973 and 1974 needles at GB-1 between the 27- and 51-month exposure periods, while premature needle casting rapidly increased at the same time:

(1) The amount of total needle tissue damage increased on the remaining and therefore healthier needles during this 12-month period. Thus, the most severely-damaged needles in 1976 were cast before the 1977 study period.

(2) The 1973 and 1974 foliage prematurely cast between the 27- and 51-month exposure periods was composed mostly of healthier needles, and the most damaged remained on their respective internodes.

Currently it is our hypothesis that the first alternative is the most likely and that the remaining needles on the older internodes have increased pathologies during the 27- to 51-month exposure period. More recently, nylon netting bags with five compartments, one for each internode, have been attached to the branches of the ponderosa pine trees at four sites. The purpose of this study is to collect, over a six-month period, the prematurely cast needles from each of the five internodes retaining foliage. These premature needles will be analyzed for pathologies and sulfur and fluoride content to test our hypothesis that needles being prematurely cast are more damaged by certain pathologies and contain higher sulfur and fluoride levels than those being retained.

The results of our measurements of needle length and fascicular cross-sectional area demonstrate that sometimes there were significant differences within plots and between plots in different-aged foliage of both younger and older trees. However, these two growth characteristics may not be useful for determining chronic air pollution impacts. We noticed that lengths and cross-sectional area of needles from the chronically-polluted GB-1 site were quite similar to those from the five Colstrip sites (S-5, SE-4, E-1, SE-2, and S-3). The ponderosa pine trees at the Billings GB-1 site have been impacted by substantial amounts of phytotoxic gases since about 1967 (Montana DHES, 1976), when the 180 MW coal-fired power plant went on-line. If the pines at this site, after ten years of fumigation with damaging levels of HF and SO_2, are still producing needles with the same general lengths and cross-sectional areas as needles from pristine sites, these two growth characteristics may not be of use for determination of chronic air pollution damage. However, these types of needle measurements may be useful in studies of areas subject to acute or high levels of chronic air pollution impacts (damage manifested within a year). For instance, Miller *et al.* (1977) reported that shorter needles were found on ponderosa pine foliage damaged by phytotoxic pollutants in the San Bernardino National Forest. Thus, use of these two growth characteristics should not be dismissed until it is determined how severely pines must be fumigated under field conditions before showing a loss of needle surface.

Fluoride levels in different-aged needles from the Colstrip sites generally remained constant over the past three years at concentrations lower than those reported in the literature as causing damage to ponderosa pine foliage. Unpublished studies by Gordon (1977) on fluoride accumulation in damaged ponderosa pine foliage collected in The Dalles, Oregon, area found fluoride concentrations in damaged needles averaging from 10 to 14 ppm, depending on the study site. In a 1977 field study of chronic fluoride pollution impact to several species of pine and fir in Columbia Falls, Montana, Carlson (1978) found concentrations of 10 to 12 ppm fluoride in damaged pine foliage.

Fluoride concentrations in different-aged foliage from BNW-1 and BNW-3 were slightly higher than levels found in foliage from the more distant Colstrip sites, SE-2 and S-5. However, fluoride levels alone do not appear to be the cause of the increased needle pathologies at BNW-1 and BNW-3.

Fluoride levels in fascicular sheaths from all three BNW sites were substantially higher than those found at the more distant Colstrip ponderosa pine sites. It is difficult to determine the source of these elevated fluoride levels because of the large amount of coal dust in the immediate Colstrip vicinity where the BNW sites are located. The utility companies operating the two power plants at Colstrip have been cited by the EPA for violations of particulate level standards at this operation. Analyses of some of the coal being mined at Colstrip showed fluoride concentrations ranging from approximately 30 to 80 ppm (Montana DHES, 1974), less than levels found in the soils of the area. However, substantial amounts of fine particulate coal dust probably become airborne in the area during blasting in the open pit mines. Thus, we are not currently attributing the elevated fluoride levels found in the foliage and the fascicular sheaths at the BNW sites to either power plant emissions or coal dust from mining operations, because it is more likely that both contribute to the increase.

The elevated fluoride levels found in both needles and fascicular sheaths from the Billings site were consistently and surprisingly high during the last two years. Total fluoride emissions from the four stationary sources in a 15-mile radius of GB-1 (three oil refineries and one 180 MW coal-fired power plant) are approximately 90 pounds per day (Montana DHES, 1974, 1977). That the fluoride concentrations in the ambient air of this site are causing a portion of the foliar damage to pines is without doubt. However, total SO_2 emissions from the stationary sources in the Billings area are approximately 57 tons per day (Montana DHES, 1974) or 2.4 tons per hour, and this gas also is impacting and accumulating in the pine foliage at the GB-1 site.

What constitutes a "normal sulfur concentration" in ponderosa pine foliage from a pristine area has been an issue of some debate in the literature on the subject. As previously mentioned, Katz and McCallum (1939) reported in an extended field study that the mean sulfur levels in ponderosa pine foliage from Summerland, B.C., were 600 to 700 ppm; three-year-old foliage from what he considered a control area 90 miles downwind from Trail, B.C., contained levels as high as 1,500 ppm.

Sulfur levels in pine foliage from the more distant Colstrip sites remained extremely consistent during the last three years, and it is difficult to accept a level of 1,500 ppm sulfur in ponderosa pine as typical on foliage from a true control site. This belief is strengthened by sulfur concentrations we found in current and older pine needles and fascicular sheaths collected from GB-1 (Figures 5.62 and 5.63) and the findings of Carlson (1974) in a field study on damaged Douglas-fir and ponderosa pine foliage around a Kraft pulp and paper mill here in Missoula, Montana. Unlike fluoride, low ambient sulfur concentrations taken in by pine foliage can be translocated out of this tissue as sulfate (Faller *et al.*, 1970). Fluoride, on the other hand, migrates to the edges of leaf tissues (Compton and Remmert, 1960; Jacobson *et al.*, 1966; Tourangeau *et al.*, 1977) where it accumulates excessively in comparison to whole leaf tissues. It is not known whether these elevated sulfur levels in older foliage (1972-1976) remained fairly constant at around 1,300 ppm because of: (1) Translocation of sulfur out of the leaf tissue or (2) needles with the most elevated levels being prematurely cast. We strongly suspect that this second supposition is applicable to areas where severe chronic and acute fumigation damage is occurring, but sulfate translocation probably explains the uniform sulfur levels at the Billings site. This supposition will be studied during the 1978 and 1979 study period using special needle catch traps to selectively collect samples of different-aged needles being prematurely cast from the internodes of trees at both the Billings and Colstrip sites.

After quantifying and studying the pine foliage growth/health/damage characteristics which occurred during the last three years, we have prepared a tentative conceptual model (Figure 5.64) to explain the occurrence rates of low level chronic air pollution symptoms such as necrosis, needle retention, and fluoride and/or sulfur accumulation. The model will be tested on the results of 1978 and 1979 studies, which will include data obtained from analyses of selectively-collected, prematurely-cast needles from polluted and pristine areas in southeastern Montana. This conceptual model is based on three major hypotheses, each of which is dependent on the others:

(1) Regardless of the cause(s) of needle necrosis or damage, when the entire surface area of a needle becomes more than 40% necrotic (38% to 50%), it will be cast from the stem during the year that level of necrosis occurs.

(2) If the needle damage is being caused by the accumulation of low, chronic levels of phytotoxic gases, such as fluoride and/or SO_2 in the foliage, prematurely cast needles will have accumulated more elevated levels of one or both of these elements than the less-damaged foliage retained on the same internode.

(3) There will be a reduction in certain necrotic symptoms, such as mottle and basal scale, on needles being retained on older internodes. The amount of other necrotic symptoms, such as basal necrosis and tip necrosis, will not decrease significantly on that same foliage.

These three tentative hypotheses were the result of our studies of trends in ranks, as well as raw data, of needle pathologies and sulfur and/or fluoride levels in different-aged foliage collected over the last three years from both "pristine" and polluted sites (S-3 and SE-4 vs GB-1 and BNW-3).

Data from past field studies carried out by Katz and McCallum (1939) and McGovern and Balsillie (1973) were utilized to test one of these hypotheses. Since none of the investigating teams presented any quantification of needle pathologies, the only hypothesis which could be tested was that sulfur levels in older needles being retained on internodes in areas of chronic air pollution would be less than levels in needles from the same year which were prematurely cast from internodes. Utilizing sulfur levels found

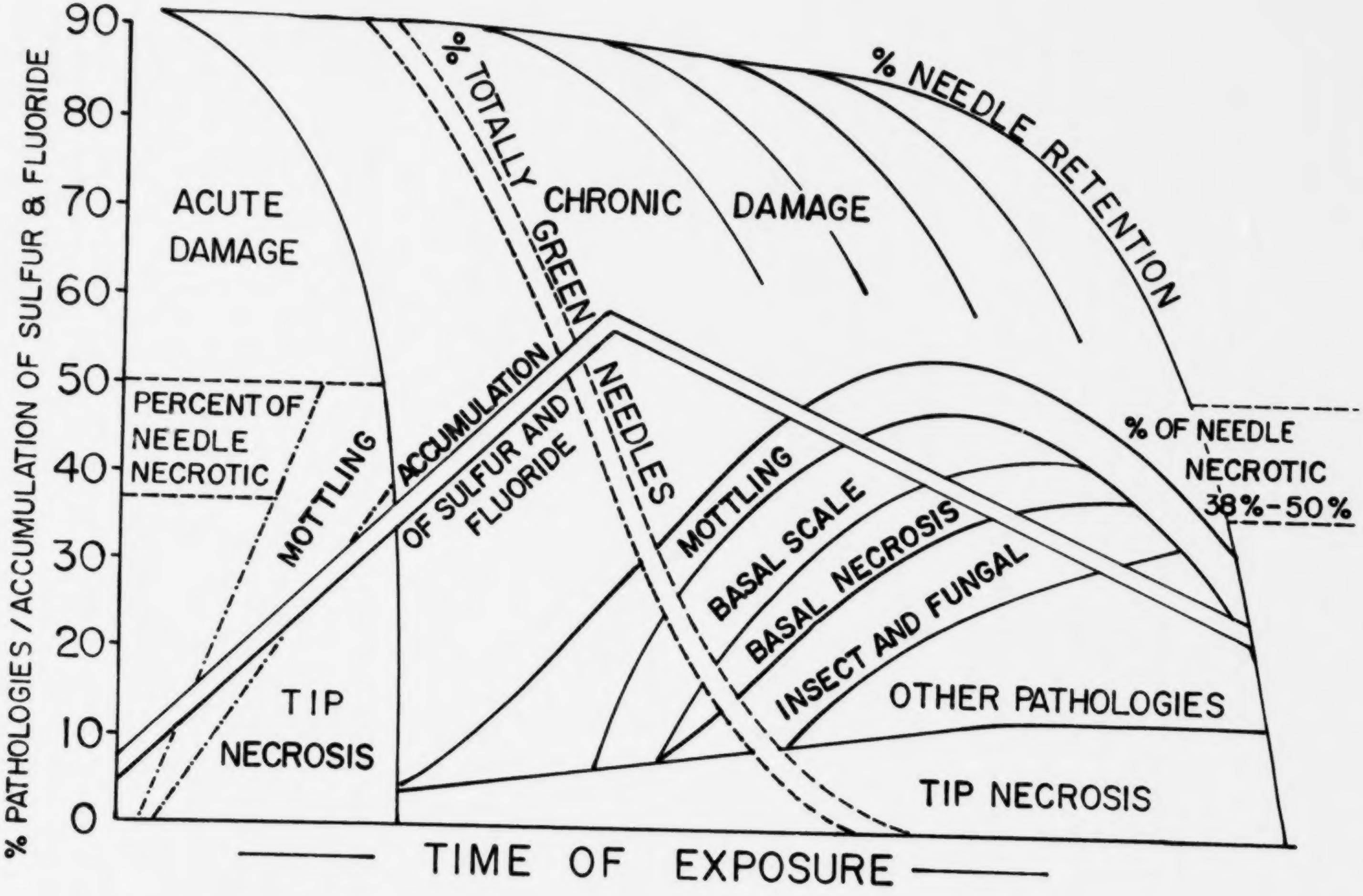


Figure 5.64. Occurrence rates of low level chronic air pollution symptoms.

in three years of pine foliage collected at increasing distances from Trail, B.C., for three consecutive years (1934-1936) by Katz, one can plot the sulfur accumulation data for any given year by increasing distance (Figure 5.65), or one can utilize the sulfur accumulation levels in 1934 needles from 1934 to 1936 and plot this data on time of exposure (Figure 5.66). In Figure 5.65, foliage at the six-mile collection site was cast somewhere between the 17th and 29th month collecting periods. Only two years of sulfur data is plotted, and no major trend in sulfur accumulation in the different-aged needles is apparent. However, when sulfur levels in 1934 foliage are plotted over the three-year study period (Figure 5.66), the trend indicates a reduction of sulfur in 1934 needles between the 17th and 29th month of exposure. This reduction in sulfur might be caused by translocation out of the older leaves to the stems and roots, and studies on this possibility should be undertaken. However, currently we suspect that this reduction in 29-month exposed needles was due to premature needle casting of those needles with the most elevated sulfur levels.

The McGovern and Balsillie (1973) studies included the collection and analysis of only two years (current and one-year-old) of Jack pine foliage, and thus it is difficult to demonstrate a trend in sulfur accumulation. However, by selecting those sulfur data which demonstrate the largest change (reduction) between the current and one-year-old foliage, it is possible to not only plot the reduction in sulfur in selected samples during a 12-month period but ascertain the trends for trace metals also analyzed by McGovern and Balsillie. In Figure 5.67 are the average sulfur levels of 6- and 18-month-old (± 4 months) Jack pine foliage collected during 1971 and 1972 from six collection sites at varying distances and directions from Sudbury, Ontario. This figure also includes the levels of six metals found in the two different-aged foliage during the collecting periods. Both nickel and zinc are generally lower in older foliage while arsenic, copper, and iron increase over these 12-month periods. If the damaged needles being prematurely cast in chronically-polluted areas are those with the most elevated levels of sulfur, and the needles retained on that year's internodes therefore show lower sulfur levels, this hypothesis could be utilized and tested for toxic trace metals in evergreen conifer species, assuming the investigator collects the same year's foliage for more than two consecutive years.

Comparison of sulfur and fluoride levels in different-aged foliage from the Colstrip sites to levels in similar-aged foliage from the polluted GB-1 Billings site adequately demonstrates that the Colstrip sites are not being subjected to the kind of ambient air concentrations as the latter. The lowest levels of ambient SO_2 and HF which can cause measurable impact on ponderosa pine and other susceptible conifer or understory species has been the subject of many studies because of the need to establish protective ambient air standards for these two gases. It is not our intention at this time to suggest "safe levels" for these two gases in the ambient air. However, the data we have accumulated on fluoride and sulfur levels in pine foliage and the growth/health/damage characteristics of pine foliage collected from both pristine and chronically-polluted areas of southeastern Montana strongly suggest that short-term controlled fumigation studies or a single season field study are totally inadequate to quantify impacts of these two gases.

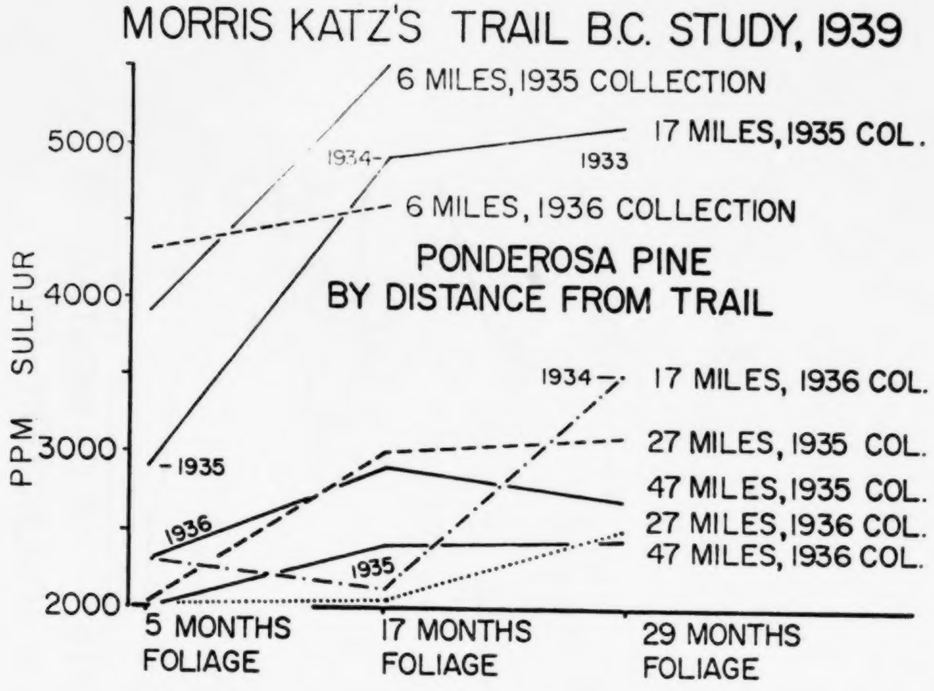


Figure 5.65. Sulfur levels in three years of ponderosa pine foliage collected at increasing distances from Trail, B.C.

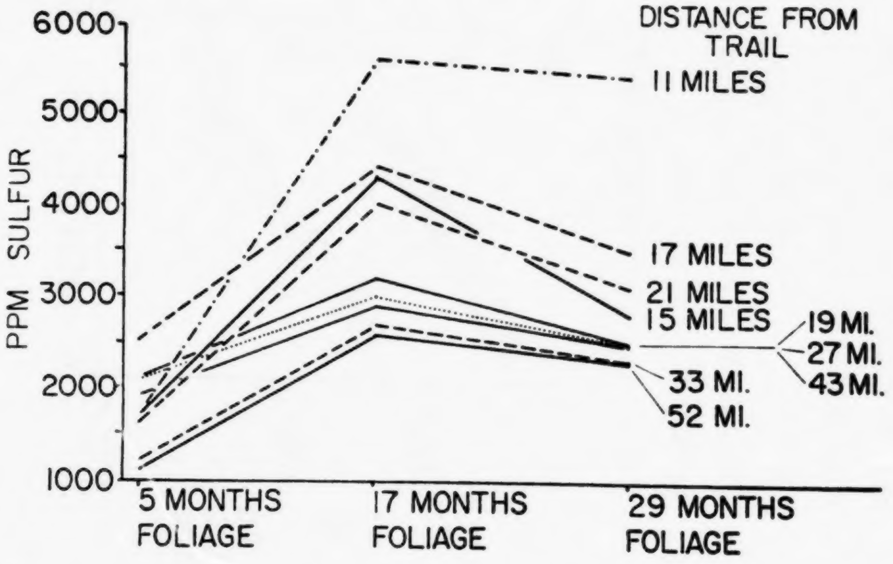


Figure 5.66. Sulfur levels in 1934 needles by foliage age and distance from Trail, B.C.

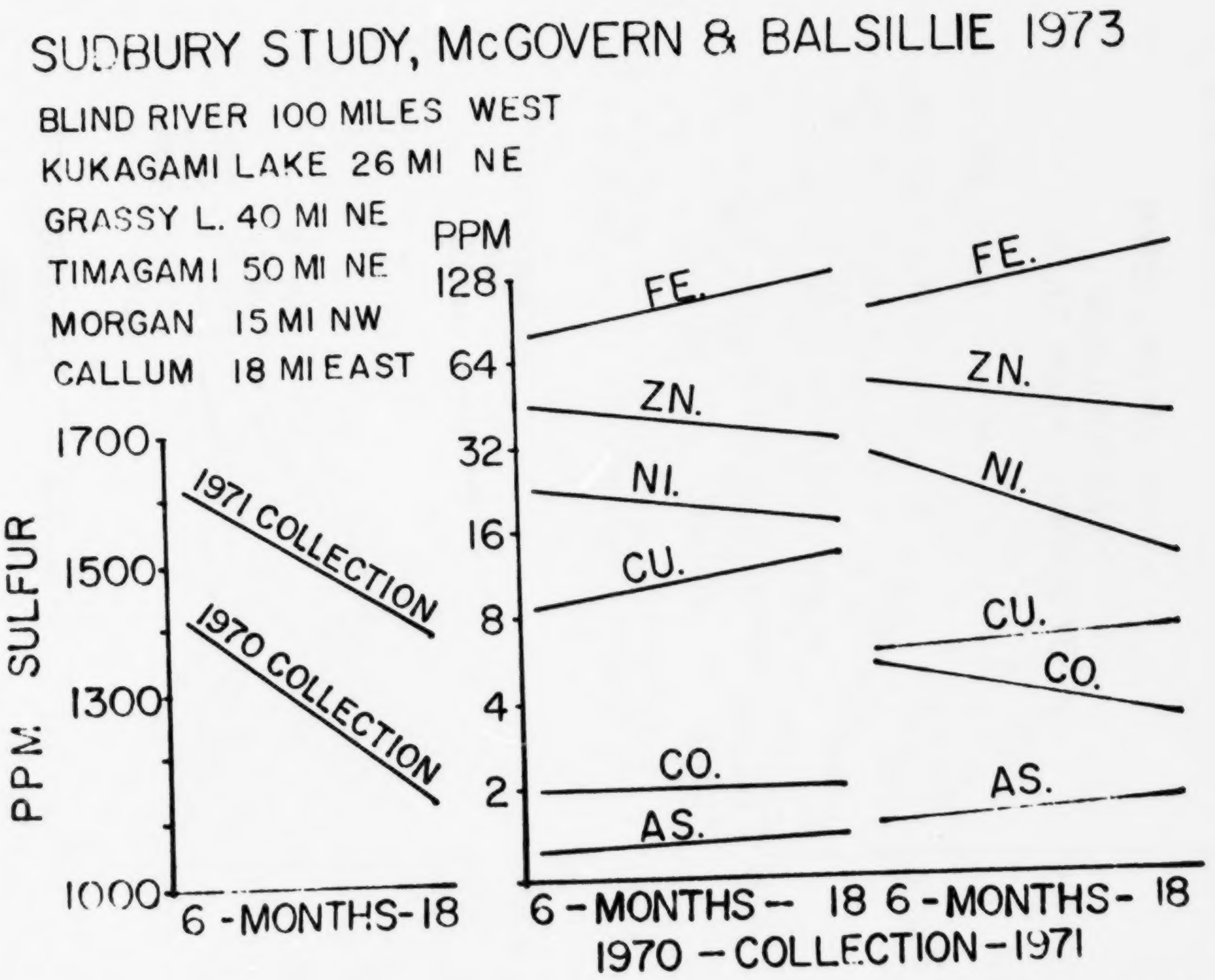


Figure 5.67. Sulfur levels in Jack pine foliage collected around Sudbury, Ontario.

For instance, Linzon (1971) used the data from a field study on vegetation damaged by emissions from two aluminum plants to conclude that 35 ppm in foliage represented an injury threshold. After reviewing the literature, McCune (1969) concluded that no foliar injury would occur at concentrations of 0.8 ppb HF in the ambient air. All of these suggestions or conclusions may be suspect after one notes that Sidhu (1977), after studying fluoride-damaged coniferous and understory species around a phosphate plant, concluded that ambient fluoride concentrations of 0.20 to 0.25 ppb should not be exceeded more than 40% of the time. In a long-term study, Facteau and Mellenthin (1976) found adverse effects to cherry orchard species in The Dalles, Oregon, area where the fluoride levels were usually less than 0.30 ppb.

Past field studies, as well as controlled fumigation chamber studies on SO_2 impacts on vegetation, also have led researchers and personnel of state and federal regulatory agencies to suggest a variety of ambient air standards to protect vegetation from SO_2 damage. For instance, Katz and McCallum (1952) concluded from their fumigation and field studies in a large area around Trail, B.C., "that vegetation will not suffer from sulfur dioxide if ground concentrations are maintained at a low level, if concentrations higher than 0.3 to 0.5 ppm do not occur, and if the duration of concentrations at the 0.3 ppm level is short in any single gas visitation." Dreisinger (1965) concluded that if the necessary environmental factors are optimum, plant injury thresholds for ambient SO_2 are 0.95 ppm for one hour, 0.55 ppm for two hours, 0.35 ppm for four hours, and 0.25 ppm for eight hours.

EPA rescinded its 24-hour secondary SO_2 ambient air standard of 0.10 ppm, as well as the annual SO_2 standard of 0.02 ppm, but maintained its three-hour standard of a maximum of 0.50 ppm, not to be exceeded once per year. These actions generally reflect the suggestions and conclusions of researchers such as Katz and McCallum (1952) and Dreisinger (1965). However, the results of our current study suggest that chronic SO_2 and HF damage to pine species occurs at substantially lower levels than suggested by most air pollution investigators. Before this study, most of the field research studies on air pollution were initiated after the pollution damage commenced. For instance, those of Katz and McCallum (1939) in Trail, B.C., where he and his co-workers conducted extensive field studies from 1930 to 1937 utilizing conifer species as a bioindicator of air pollution impacts, and those of Linzon (1958), Dreisinger and McGovern (1970, 1971), McGovern and Dreisinger (1970), and McGovern and Balsillie (1974) who carried out extended studies from 1950 until the present in the severely air pollution-impacted area of Sudbury, Ontario. There is little evidence in any of the above publications that the authors could and actually did differentiate between conifers impacted by air pollution and normal healthy growth of coniferous forests.

Another major reason we hypothesize that chronic air pollution damage to pine species occurs at low ambient levels of phytotoxic gases is that chronic air pollution-caused symptoms on pine foliage can mimic those occurring on pine foliage in pristine environments. Conifers in a pristine, healthy forest have foliage damaged by abiotic and biotic causal agents which slowly increase with the advent of low chronic air pollution. It is therefore difficult for an investigator using the typical macroscopic foliar symptoms (tip burn and mottle) to measure this subtle increase without first quantifying the "normal" levels of damage within a pristine forest. An investigator will also have difficulty separating the characteristics of a chronically-air polluted area from those of a normal, pristine area by determining "baseline concentrations" of sulfur and/or fluoride in foliage collected 50 to 100 miles from an acutely damaged area such as Sudbury, Ontario, or Trail, B.C. This, we believe, is especially true in these two areas, because the acute damage to the forest ecosystems occurred many years before any extensive investigation was initiated.

REFERENCES

Carlson, C.E. 1974. Sulfur Damage to Douglas-Fir Near a Pulp and Paper Mill in Western Montana. Report No. 74-13. USDA Forest Service, Division of State and Private Forestry, Missoula, Montana. 41 pp.

________. 1978. Fluoride Induced Impact on a Coniferous Forest Near the Anaconda Aluminum Plant in Northwestern Montana. Ph.D. Thesis, University of Montana, Missoula, Montana. 165 pp.

________, and J.E. Dewey. 1971. Environmental Pollution by Fluorides in Flathead National Forest and Glacier National Park. USDA Forest Service, Division of State and Private Forestry, Forest Insect and Disease Branch, Missoula, Montana. 57 pp.

Cobb, F.W., Jr., D.L. Wood, R.W. Stark, and J.R. Parmeter, Jr. 1968. Photochemical Oxidant Injury and Bark Beetle (Coleoptera: Scolytidae) Infestation of Ponderosa Pine. IV. Theory on the Relationships Between Oxidant Injury and Bark Beetle Infestation. Hilgardia, 39(6):141-152.

Compton, O.C., F.W. Adams, W.M. Mellenthin, S. Elliot, N. Chestnut, and D.W. Bonney. 1968. Fluorine Levels in Crops of The Dalles Area, 1965-67: Cherry, Peach, and Pine Trees and the Ambient Air. Special Report 261. Agricultural Experiment Station, Oregon State University, Corvallis, Oregon. 40 pp.

________, and L.F. Remmert. 1960. Effects of Air-borne Fluorine on Injury and Fluorine Content of Gladiolus Leaves. Proc. Am. Soc. Hort. Sci., 75:663-675.

________, L.F. Remmert, and W.M. Mellenthin. 1960. Comparison of Fluorine Levels in Crops Before and After Aluminum Factory Operations in The Dalles Area. Miscellaneous Paper 95. Agricultural Experiment Station, Oregon State College, Corvallis, Oregon. 27 pp.

Conover, W.J. 1971. Practical Nonparametric Statistics. John Wiley & Sons, Inc., New York. 462 pp.

Crecelius, E.A., L.A. Rancitelli, and S. Garcia. 1978. Power Plant Emissions and Air Quality. In: Potential for Gaseous and Heavy Metal Contamination from Energy Extraction Processes in the Northern Great Plains and the Consequent Uptake and Turnover in Range Ecosystems. ERDA Annual Report, Activity RX-02-03. Ames Laboratory, Iowa State University, Ames, Iowa. pp. 9-33.

Dreisinger, B.R. 1965. Sulphur Dioxide Levels and the Effects of Gas on Vegetation Near Sudbury, Ontario. Presented at the 58th Annual Meeting of the Air Pollution Control Association, Toronto, Ontario.

________, and P.C. McGovern. 1970. Sulphur Dioxide Levels and Resultant Injury to Vegetation in the Sudbury Area During the 1969 Season. Department of Energy and Resources Management, Sudbury, Ontario. 33 pp.

________. 1971. Sulphur Dioxide Levels and Vegetation Injury in the Sudbury Area During the 1970 Season. Department of Energy Resources and Management, Sudbury, Ontario. 38 pp.

Evans, L.S., and P.R. Miller. 1972. Ozone Damage to Ponderosa Pine: A Histological and Histochemical Appraisal. Am. J. Bot., 59:297-304.

Facteau, T.J., and W.M. Mellenthin. 1976. Fluoride Investigations in The Dalles Area 1968-1974. Technical Bulletin 132. Agricultural Experiment Station, Oregon State University, Corvallis, Oregon. 56 pp.

Faller, von H., K. Herwig, and H. Kuhn. 1970. Assimulation of Sulphur Dioxide ($S^{35}O_2$) from the Air. I. Influence on the Plant Yield. Plant and Soil, 33:177-191.

Farrier, M.H. 1972. Report on Insects and Mites in Relation to the Long-Short Needle Syndrome of Scotch Pines and Their Abundance in Christmas Tree Plantations in Western Maryland and Northern West Virginia. Unpublished report prepared for the Virginia Electric and Power Company Richmond, Virginia. 57 pp.

Gordon, C.C. 1974. Environmental Effects of Fluoride: Glacier National Park and Vicinity. EPA-908/1-74-001, U.S. Environmental Protection Agency, Denver, Colorado. 150 pp.

________. 1977. Accumulation of Fluoride in Vegetation Growing in the Vicinity of the Martin Marietta Aluminum Plant, The Dalles, Oregon. Unpublished report to the orchard growers of The Dalles, Oregon, prepared at the Environmental Studies Laboratory, University of Montana, Missoula, Montana. 10 pp.

________, C.E. Carlson, and P.C. Tourangeau. 1976. A Cooperative Evaluation of Potential Air Pollution Injury and Damage to Coniferous Habitats on National Forest Lands Near Colstrip, Montana. Report No. 76-12. USDA Forest Service, Northern Region, Missoula, Montana, and Environmental Studies Laboratory, University of Montana, Missoula, Montana. 89 pp.

________, P.C. Tourangeau, and P.M. Rice. 1978a. Investigation of the Impact of Coal-Fired Power Plant Emissions upon the Disease/Health/Growth Characteristics of Ponderosa Pine-Skunkbush Ecosystems and Grassland Ecosystems in Southeastern Montana. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Third Interim Report, Colstrip, Montana, December, 1977, E.M. Preston and R.A. Lewis, eds. EPA-600/3-78-021, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 65-139.

________. 1978b. Potential for Gaseous Contamination from Energy Extraction Processes in the Northern Great Plains. In: Potential for Gaseous and Heavy Metal Contamination from Energy Extraction Processes in the Northern Great Plains and the Consequent Uptake and Turnover in Range Ecosystems. ERDA Annual Report, Activity RX-02-03. Ames Laboratory, Iowa State University, Ames, Iowa. pp. 53-137.

Jacobson, J.S., L.H. Weinstein, D.C. McCune, and A.E. Hitchcock. 1966. The Accumulation of Fluorine by Plants. J. Air Poll. Contr. Assoc., 16(8):412-417.

Katz, M., and A.W. McCallum. 1939. Effects of Sulfur Dioxide on Vegetation. NRC No. 815. National Research Council of Canada, Ottawa, Ontario. 447 pp.

________. 1952. The Effects of Sulfur Dioxide on Conifers. In: Air Pollution Proceedings, U.S. Technical Conference on Air Pollution, Louis McCade, ed. New York, Toronto, London. pp. 84-96.

Keifer, H.H., and J.L. Saunders. 1972. *Trisetacus campnodus*, n. sp. (Acarina: Eriophyidae), Attacking *Pinus sylvestris*. Ann. Entomol. Soc. Am., 65(1):46-49.

Linzon, S.N. 1958. The Influence of Smelter Fumes on the Growth of White Pine in the Sudbury Region. Ontario Department of Lands and Forests, Ontario Department of Mines, Toronto, Ontario. 45 pp.

________. 1971. Fluoride Effects on Vegetation in Ontario. In: Proceedings of the Second International Clean Air Congress, H.M. Englund and W.T. Berry, eds. Academic Press, New York. pp. 277-289.

McCune, D.C. 1969. On the Establishment of Air Quality Criteria, With Reference to the Effects of Atmospheric Fluorine on Vegetation. Air Quality Monograph 69-3. American Petroleum Institute, New York. 33 pp.

McGovern, P.C., and D. Balsillie. 1973. Sulphur Dioxide (1972)--Heavy Metal (1971) Levels and Vegetative Effects in the Sudbury Area. Air Management Branch, Ontario Ministry of the Environment, Sudbury, Ontario. 50 pp.

________. 1974. Effects of Sulphur Dioxide and Heavy Metals on Vegetation in the Sudbury Area (1973). Ontario Ministry of the Environment, Sudbury, Ontario. 47 pp.

________, and B.R. Dreisinger. 1970. Sulphur Dioxide Levels in the Wawa Area During 1969. Department of Energy and Resources Management, Sudbury, Ontario. 23 pp.

Miller, P.R., R.N. Kickert, O.C. Taylor, R.J. Arkley, F.W. Cobb, Jr., D.L. Dahlsten, P.J. Gersper, R.F. Luck, J.R. McBride, J.R. Parmeter, Jr., J.M. Wenz, M. White, and W.W. Wilcox. 1977. Photochemical Oxidant and Air Pollution Effects on a Mixed Conifer Forest Ecosystem--A Progress Report. EPA-600/3-77-104, Environmental Protection Agency, Corvallis, Oregon. 339 pp.

Montana Department of Health and Environmental Sciences. 1974. Annual Air Quality Data Summary for Montana for 1973, J. Gelhaus, ed. Air Quality Bureau, Environmental Sciences Division, Helena, Montana.

________. 1976. Air Quality Assessment of Colstrip, Montana, Prior to Development of Coal-Fired Power Plants, J. Gelhaus, ed. Air Quality Bureau, Environmental Sciences Division, Helena, Montana. 90 pp.

________. 1977. Personal Communication. Air Quality Bureau, Environmental Sciences Division, Helena, Montana.

________. 1978. Annual Air Quality Data Summary for Montana for 1977, J. Gelhaus, ed. Air Quality Bureau, Environmental Sciences Division, Helena, Montana. 89 pp.

Northern Cheyenne Tribe. 1976. The Northern Cheyenne Air Quality Redesignation Report and Request. Northern Cheyenne Tribe, Inc., Lame Deer, Montana. 190 pp.

Sidhu, S.S. 1977. Fluoride Levels in Air, Vegetation, and Soil in the Vicinity of a Phosphorus Plant. Presented at the 70th Annual Meeting of the Air Pollution Control Association, Toronto, Ontario, June 20-24. 16 pp.

Sokal, R.R., and F.J. Rohlf. 1969. Biometry. W.H. Freeman & Company, San Francisco, California. 776 pp.

Tourangeau, P.C., C.C. Gordon, and C.E. Carlson. 1977. Fluoride Emissions of Coal-Fired Power Plants and Their Impact Upon Plant and Animal Species. Fluoride, 10(2):47-62.

Treshow, M., F.K. Anderson, and F. Horner. 1967. Responses of Douglas-Fir to Elevated Atmospheric Fluorides. For. Sci., 13(2):114-120.

Weber, D., and C. Olson. 1978. Preliminary Data on Colstrip Stack Plume Intercepts Measured at Hay Coulee, 8-24-77 to 1-12-78. Presented at the EPA Colstrip Coal-Fired Power Plant Project Workshop, Corvallis, Oregon, January 17-19.

Wood, F.A., and S.P. Pennypacker. 1975. Evaluation of the Effects of Air Pollution on Vegetation in the Mt. Storm, West Virginia-Oakland, Maryland area. Presented at the 68th Annual Meeting of the Air Pollution Control Association, Boston, Massachusetts, June 15-20.

APPENDIX 5.1

PROCEDURE FOR THE DETERMINATION OF AVERAGE RANKS

Appendix Figure 5.1 is a reproduction of Figure 4.7 from the Third Interim Report, which shows the mean values and 95% confidence intervals for percent basal necrosis in 1972, 1973, and 1974 foliage, upper and lower crown positions, from plots E-1 and SE-2 (now called sites) collected in 1975. This figure was prepared after the data were coded to the arcsin for percentages.

Appendix Figure 5.2 is a histogram of 120 observations used in the preparation of Appendix Figure 5.1. The data were not coded to the arcsin for percentages before Appendix Figure 5.2 was prepared. The arcsin transformation would not make these data more Gaussian because of the large number of zeros. The number of variates in the class intervals in this figure are shown below in Appendix Table 5.1.

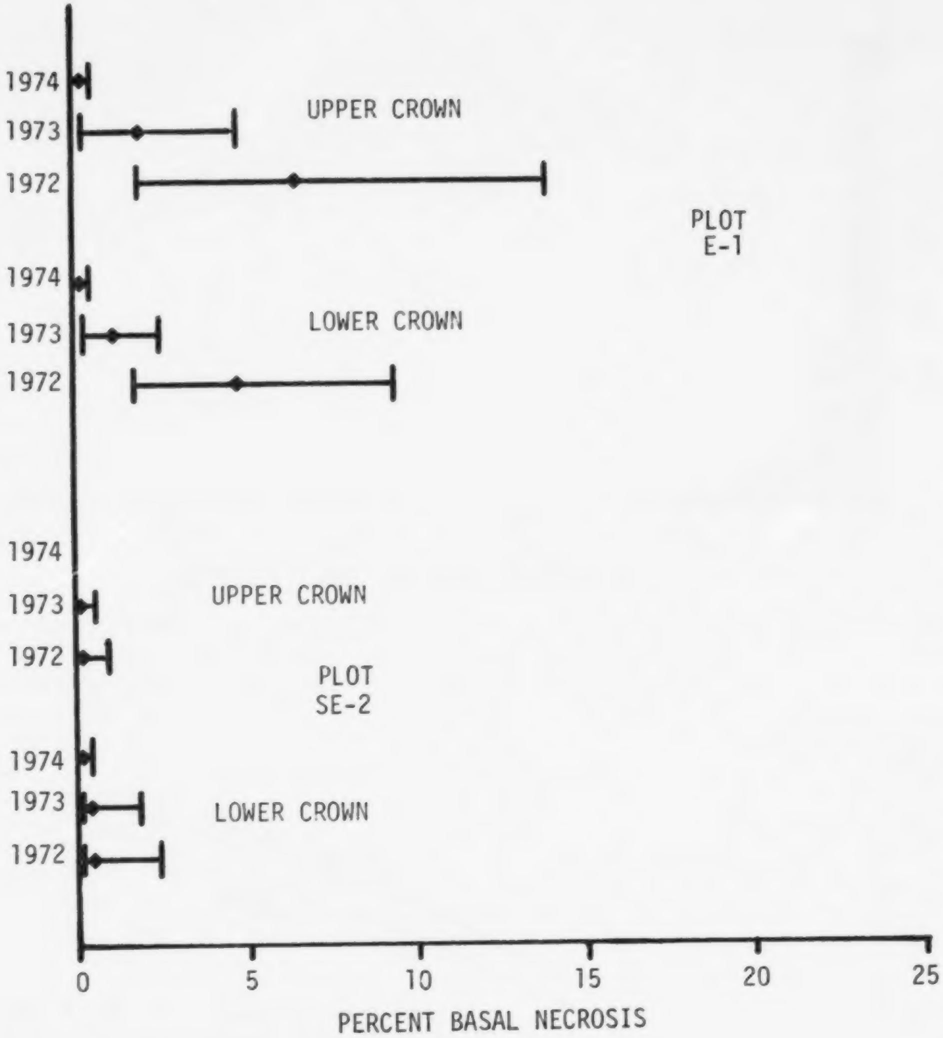
APPENDIX TABLE 5.1. NUMBER OF VARIATES FOR CLASS INTERVALS FOR APPENDIX FIGURE 5.2

Class Interval	Number of Variates
0	71
2	24
4	7
6	7
8	2
10	3
12	1
14	2
18	2
32	1

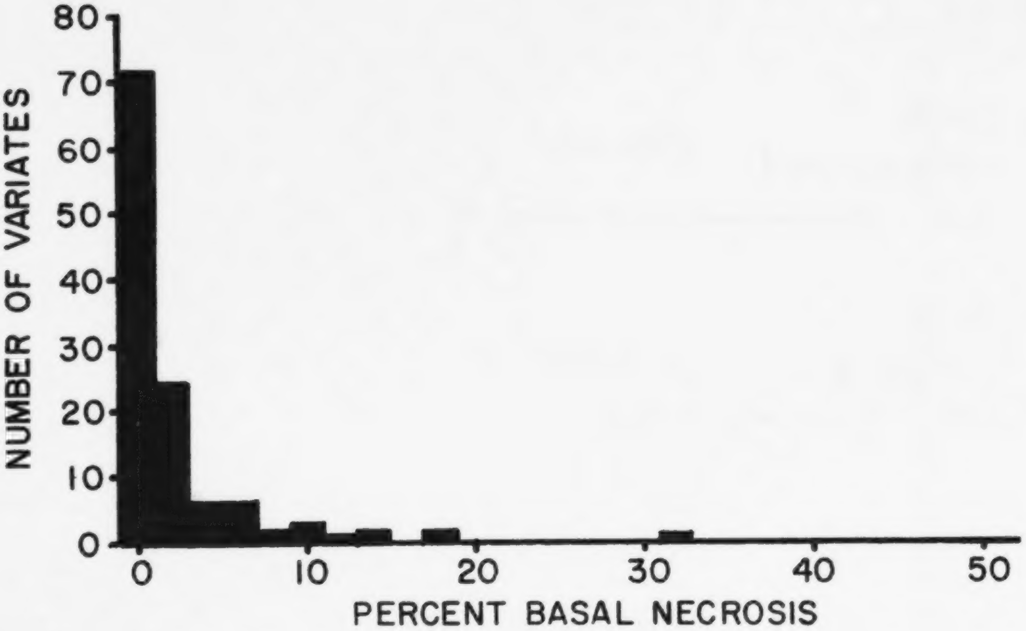
Appendix Figure 5.3 shows the average ranks for percent basal necrosis in the 1972, 1973, and 1974 years of foliage, upper and lower crown positions, for sites E-1 and SE-2, 1975 collection. While Appendix Figure 5.1 shows the mean values and 95% confidence intervals for these same data after arcsin coding, Appendix Figure 5.3 shows these data after ranking of the uncoded variates.

The procedure for the determination of the average ranks as shown in Appendix Figure 5.3 involves ordering all of the 120 variates from lowest value to highest ($X_1, X_2, X_3 \ldots X_{120}$). The variates are then ranked from 1 to n ($R_1, R_2, R_3 \ldots R_n$). The ranked data are then segregated into the original samples and the average rank computed for each sample by $\frac{\left(\sum^{n_i} R\right)_i}{n}$

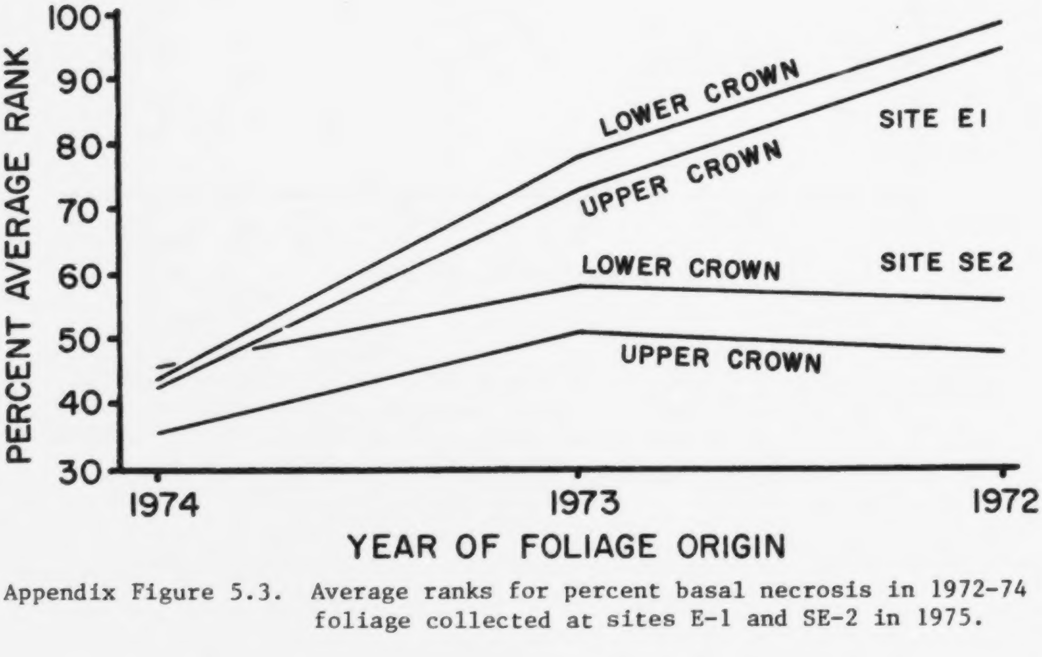
Appendix Table 5.2 shows the variates, their ranks, and the average rank for the data shown in Appendix Figure 5.3.



Appendix Figure 5.1. Mean values and 95% confidence intervals for percent basal necrosis in the upper and lower crowns of 1972, 1973, and 1974 foliage collected at plots (sites) E-1 and SE-2 in 1975.



Appendix Figure 5.2. Histogram of 120 variates used to compute mean values and 95% confidence intervals for percent basal necrosis in 1972-74 foliage collected at E-1 and SE-2 in 1975.



Appendix Figure 5.3. Average ranks for percent basal necrosis in 1972-74 foliage collected at sites E-1 and SE-2 in 1975.

APPENDIX TABLE 5.2. VARIATES FOR PERCENT BASAL NECROSIS AND THEIR RANKS IN UPPER AND LOWER CROWN FOLIAGE FROM SITES E-1 AND SE-2, 1975 COLLECTION (1972-74 YEARS OF FOLIAGE)

Percent Basal Necrosis	Rank		Percent Basal Necrosis	Rank	
Site E-1/Upper Crown/1972 Foliage			Site E-1/Lower Crown/1972 Foliage		
0	36.0		0	36.0	
1	77.5		1	77.5	
1	77.5		2	89.5	
4	100.5		3	97.0	
5	105.0	Average Rank	4	100.5	Average Rank
8	111.0		4	100.5	
9	112.0	97.4	6	108.5	95.7
11	115.0		9	112.5	
18	118.0		14	116.5	
31	120.0		18	118.5	
Site E-1/Upper Crown/1973 Foliage			Site E-1/Lower Crown/1973 Foliage		
0	36.0		0	36.0	
0	36.0		0	36.0	
0	36.0		0	36.0	
1	77.5		1	77.5	
2	89.5	Average Rank	1	77.5	Average Rank
2	89.5		1	77.5	
2	89.5	78.0	2	89.5	72.5
5	105.0		2	89.5	
5	105.0		3	97.0	
14	116.5		6	108.5	
Site E-1/Upper Crown/1974 Foliage			Site E-1/Lower Crown/1974 Foliage		
0	36.0		0	36.0	
0	36.0		0	36.0	
0	36.0		0	36.0	
0	36.0		0	36.0	
0	36.0	Average Rank	0	36.0	Average Rank
0	36.0		0	36.0	
0	36.0	42.9	0	36.0	44.3
0	36.0		0	36.0	
0	36.0		1	77.5	
5	105.0		1	77.5	

(continued)

APPENDIX TABLE 5.2. VARIATES FOR PERCENT BASAL NECROSIS AND THEIR RANKS IN UPPER AND LOWER CROWN FOLIAGE FROM SITES E-1 AND SE-2, 1975 COLLECTION (1972-74 YEARS OF FOLIAGE)

(continued)

Percent Basal Necrosis	Rank		Percent Basal Necrosis	Rank	
Site SE-2/Upper Crown/1972 Foliage			Site SE-2/Lower Crown/1972 Foliage		
0	36.0		0	36.0	
0	36.0		0	36.0	
0	36.0		0	36.0	
0	36.0		0	36.0	
0	36.0	Average Rank	0	36.0	Average Rank
0	36.0		0	36.0	
0	36.0	48.6	0	36.0	56.0
0	36.0		2	89.5	
3	97.0		5	105.0	
4	100.5		10	114.0	
Site SE-2/Upper Crown/1973 Foliage			Site SE-2/Lower Crown/1973 Foliage		
0	36.0		0	36.0	
0	36.0		0	36.0	
0	36.0		0	36.0	
0	36.0		0	36.0	
0	36.0	Average Rank	0	36.0	Average Rank
0	36.0		0	36.0	
0	36.0	50.5	1	77.5	58.3
1	77.5		2	89.5	
2	89.5		2	89.5	
2	89.5		7	110.0	
Site SE-2/Upper Crown/1974 Foliage			Site SE-2/Lower Crown/1974 Foliage		
0	36.0		0	36.0	
0	36.0		0	36.0	
0	36.0		0	36.0	
0	36.0		0	36.0	
0	36.0	Average Rank	0	36.0	Average Rank
0	36.0		0	36.0	
0	36.0	36.0	0	36.0	45.5
0	36.0		0	36.0	
0	36.0		1	77.5	
0	36.0		2	89.5	

SECTION 6

HONEYBEES AND OTHER INSECTS AS INDICATORS OF POLLUTION IMPACT FROM THE COLSTRIP POWER PLANTS

J. J. Bromenshenk

ABSTRACT

In 1977, I continued post-operational assessments of the distribution and magnitude of impacts from the Colstrip power plants using terrestrial insects as biological indicators of environmental quality. I concentrated on insect species and systems that from my baseline and comparative studies appeared to be particularly sensitive or susceptible to pollution stress and are important or "key" species, both ecologically and economically, of ecosystems of southeastern Montana. These included: (1) Pollinators (honeybees) which are bioaccumulators or biocollectors of noxious substances from their surroundings and which may be very susceptible to poisoning or less obvious types of damage from these materials; (2) insects on ponderosa pines, with the emphasis on the scale insect *Matsucoccus secretus* Morrison, which resides under the sheath of pine needles, often occurs proximal to areas of needle injury, and belongs to a genus of insects reported to proliferate in areas subjected to pollution stress, and (3) ground-dwelling beetles, saprophagic and predatory insects, whose abundance may be adversely affected by exposure to long-term, low levels of sulfur oxides (see Section 18).

Honeybees proved to be efficient scavengers of substances other than the fluorides which I originally examined. Significant post-operational changes of fluoride levels in bee tissues at sites near Colstrip compared to controls and baseline levels continued to be observed. Pilot studies indicated that bees also accumulate arsenic and possibly zinc and radionuclides, both naturally-occurring and anthropogenic forms. Bioassays for other trace elements are ongoing. Levels of radionuclides should provide indices to the scavenging efficacy of bees in terms of the particle size of pollutants.

Several lines of evidence supported the hypotheses that insects can be used to detect and to elucidate effects of environmental pollutants on terrestrial ecosystems and that impacts on insect systems may induce changes in fundamental ecosystem components and processes.

INTRODUCTION

During the 1977 growing season, I continued post-operational assessments of the distribution and the magnitude of impacts from the Colstrip power plants using terrestrial insects, primarily honeybees, as biological indicators of environmental quality. In 1974, I hypothesized that methods could be developed to predict the bioenvironmental impacts of coal-fired power plants before damage occurred, based on the use of indicator species of insects as early warning systems and as continuous monitors of atmospheric pollutants. The Colstrip environmental assessment project was unique because it provided both an opportunity to conduct baseline studies in a relatively pristine area before the power plants began operation (in 1975 and 1976) and to monitor post-impact changes in ecosystems of the Northern Great Plains.

Following my preliminary investigations (Bromenshenk, 1976, 1978a), I directed my studies towards those insect species and systems which appeared to be particularly sensitive or susceptible to pollution and which were important ecologically and economically to the grasslands and timber stands of southeastern Montana. These included: (1) Pollinators, especially honeybees, which gather noxious substances from their environment and which may be susceptible to poisoning from these substances; (2) insects living on ponderosa pine, with emphasis on the scale insect *Matsucoccus secretus* Morrison, which resides under the sheath of pine needles, which often was observed at sites with trees showing basal necrosis or burn of needles such as is typical of acid rain injury, and which may increase in numbers in areas subjected to pollution stress (based on studies of *M. pini* by Siewniak, 1971), and (3) ground-dwelling beetles, including important saprophagic and predacious species, which appear to be affected by chronic, low-level concentrations of SO_2 (covered in Section 18).

I continued to characterize and study insect-induced injuries to pine foliage through histopathological examinations in order to delineate insect pathologies from other biotic (fungi, viral, bacterial) and abiotic (frost, drought, phytotoxins, acid rain) factors. Results for 1977 from these studies and those concerning the *Matsucoccus* scale were, for the most part, incomplete at the time of preparation of this report (May, 1978) and as such shall appear in a later report. The bulk of the work on damage to pines by insect pests is included in Section 5 of this report and concentrates on damage rather than absolute insect population densities.

I shall concentrate here on the results of my honeybee studies during 1977 in the vicinity of Colstrip. A detailed account of the rationale for the insect work, baseline data, methods, and the selection criteria for subsequent investigations appeared in the Third Interim Report (Bromenshenk, 1978a).

Honeybees forage large areas (about 10,000 acres, according to Toshkov *et al.*, 1973), visit innumerable flowers, and travel tremendous distances. The gathering of one pound of honey necessitates a total flight distance equivalent to three orbits around the earth (Crane, 1975), and a honeybee colony may gather as much as 200 pounds of honey during a single season. Honeybees collect water for drinking, for dilution of food, and for evaporative cooling of their hives. They forage for pollen, nectar, and honeydew to be used as food, and they search for resin to seal and weather-proof their hives. Honeybees are behaviorally and morphologically adapted for the collection of these materials, but they do not seem to be able to screen out other substances that settle on the surfaces of plants, on the bees themselves, or which are adsorbed on nectar and honeydew or absorbed in water. Since their respiratory passages communicate directly to the air and lack protective linings such as mucous membranes, bees inhale fine dusts, mists, or gases; although hairs surrounding the spiracles probably filter out larger particles.

Honeybees are biological magnifiers of contaminants. Pollutants accumulate on or in their body tissues. In general, the levels of contaminants are highest in the tissues of adult bees and are much lower in pollen, nectar, floral parts, or water (Lillie, 1972; Bromenshenk, 1978a, 1978b; Bromenshenk and Gordon, 1978).

Honeybees are social insects and can be managed. Therefore, they provide in quantity materials for sampling (*i.e.*, bees, pollen, and nectar or honey). By sampling vegetation and water in the vicinity of a bee colony and by using physico-chemical measurements of air quality, one can obtain a very comprehensive profile of the environmental quality of the area and examine transportation routes of contaminants through honeybee systems. Presumably, honeybees should serve as a model for the passage of these materials through other pollination systems.

Although useful to man as a detector of pollutants, honeybees can be harmed by an accumulation of toxic substances. The vigor of their colonies may be affected and there may be other effects such as reductions in pollination and honeybee production (Bromenshenk, 1978a, 1978b; Debackere, 1972; Steche, 1975).

In 1974, I initiated studies of the concentrations of sulfur and fluoride in honeybee systems. I chose honeybees based on their reported sensitivity to industrial pollution and on their ecological importance as pollinators. Beekeeping is a major agricultural industry in Montana, and 6,600 colonies are located in the Colstrip vicinity. In the Third Interim Report, which included results of my studies from 1974 through 1976, I concluded that honeybees are excellent detectors of fluoride accumulation and that they might also be excellent detectors of other anthropogenic trace elements (Bromenshenk, 1978a). Baseline levels of fluoride in bees near Colstrip in 1974 and 1975 averaged about 8 ppm. The concentration of fluoride in bees from apiaries downwind from the Colstrip power complex increased as much as twofold the year after the two 350 MW power plants began operations. Fluoride in bees from apiaries located upwind or at distances greater than 40 km from the power plants did not increase. The increase of fluoride levels in or on the bodies of bees did not correlate with the fluoride content of water supplies or pollen. In fact, pre- and post-operational levels of fluoride in water and pollen were not significantly different.

Water supplies are a possible source of fluoride. Both before and after operation of the Colstrip power plants, body tissue levels of fluorides in bees from a beeyard near Rosebud, Montana, averaged as much as 200 ppm, a level greater than that in bees we sampled near an aluminum reduction facility in northwestern Montana. Artesian well water which was used by the bees at this location had 20 to 50 times as much fluoride as the surface waters at other apiaries in the Colstrip area. Fluoride levels in pollen and floral parts collected at this site were not elevated over levels from other sites.

In my 1974 through 1976 studies, I was not able to detect significant differences in sulfur in bee tissues either near Colstrip or in bees maintained at the ZAPS SO_2 fumigation plots. Relatively high and variable baseline sulfur levels (3,500 to 4,800 ppm) in bee tissues (probably present in protein bonds) presumably masked any changes attributable to small incremental increases of anthropogenic sulfurs in the surroundings (Hillmann, 1972) of the honeybees.

MATERIALS AND METHODS

In 1977, bees were sampled from 16 apiaries (see Figure 6.1) during late June and again in mid-September. Most hives could not be sampled until June at the earliest because "migrant" colonies were brought in by the beekeeper from California in early May and then stockpiled along Rosebud Creek about 35 km northeast of Colstrip until they could be distributed throughout the region. September was the latest that most of the apiaries could be sampled because most of the hives were taken back to California in October.

A few hives have been left by a Rosebud county beekeeper each winter at 12 sites near Colstrip. These were used for comparison to the migrant colonies. Apiaries located south and east of the town of Ashland are owned by two beekeepers who do not practice migratory beekeeping. These colonies have never been taken out of the region and as such could be used for comparison to the transported colonies and as monitors for long distance transport of pollutants. Approximately 300 bees (30 gm wet weight) were obtained at the entrance to each hive by using a high velocity, battery-powered, acrylic plastic vacuum apparatus, which drew the bees directly into plastic jars. All samples were immediately frozen under dry ice and stored in plastic Whirl Paks® at -10°C until they could be analyzed. Three to five grams of pollen were obtained from each hive by removing pellets of pollen from honeycombs with a plastic pick. Pollen was stored in plastic vials at room temperature until analyzed. Honey was taken from each hive by drawing a plastic vial across recently capped honeycombs and was stored in the same containers at -10°C.

Ten individual hives were sampled for bees, pollen, and honey at each apiary location. Also, a pooled sample of approximately 1,000 bees (100 gm wet weight) was obtained by sampling bees from every hive at each location (15 to 50 hives). Pooled samples provided both an average sample from each location and sufficient quantities of material for tests, such as quality

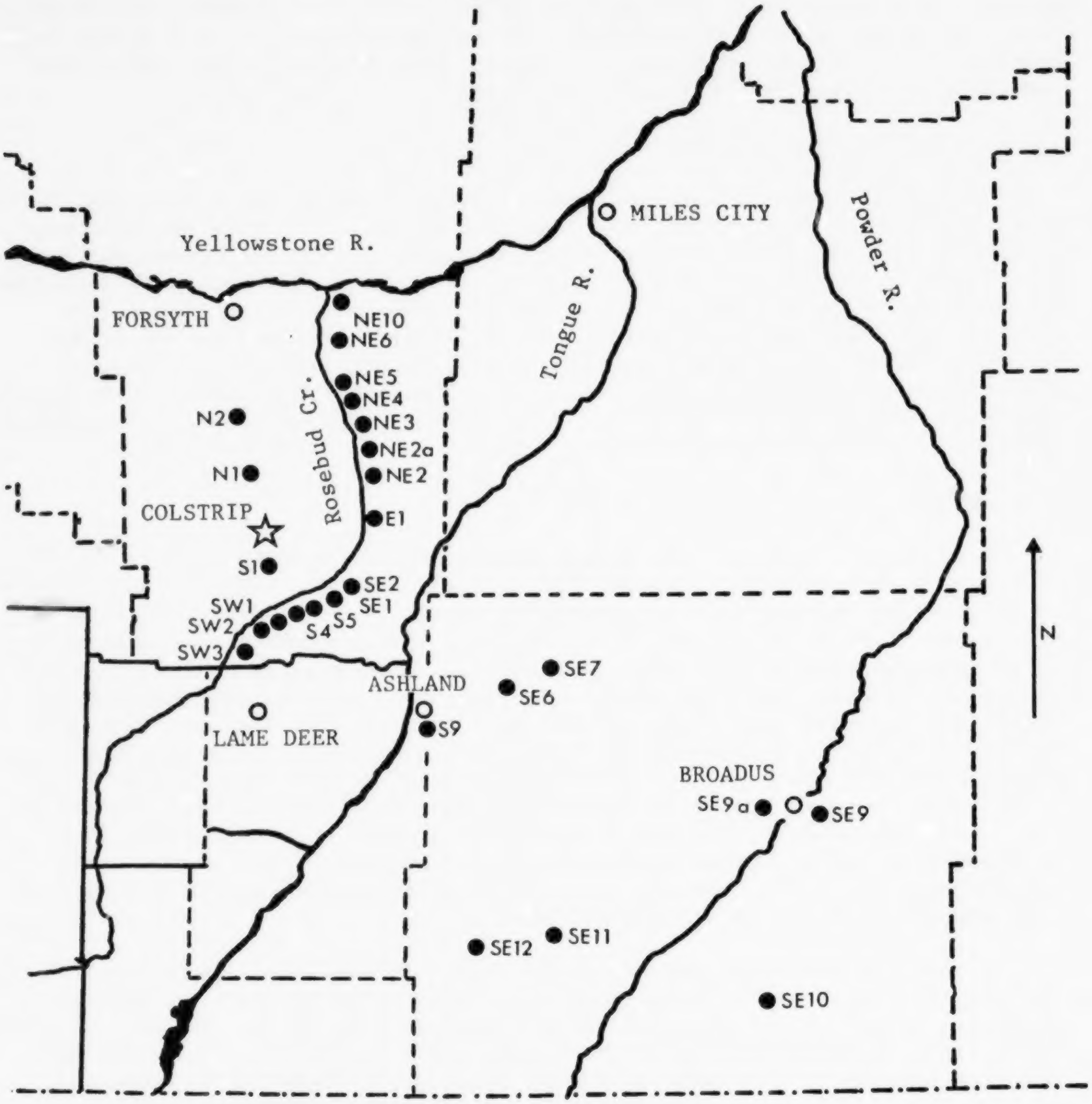

Yellowstone R.
MILES CITY
FORSYTH
NE10
NE6
NE5
NE4
NE3
NE2a
NE2
E1
N2
N1
COLSTRIP
S1
SE2
SE1
SW1
SW2
SW3
S4
S5
Rosebud Cr.
Tongue R.
Powder R.
N
ASHLAND
SE7
SE6
S9
LAME DEER
BROADUS
SE9a
SE9
SE12
SE11
SE10

Figure 6.1. Colstrip study area; locations of apiary sampling sites.

assurance tests and determinations for radionuclides, which require large numbers of bees.

Since I did not find appreciable levels of insecticides in honeybees during 1974 through 1976 (Bromenshenk, 1978a), I did not attempt to determine pesticide levels in the 1977 samples. If an unusual mortality or a report of heavy pesticide usage had occurred, then I would have performed these time-consuming and relatively expensive analyses.

Fluoride and Sulfur Analyses

Before being tested for fluoride and sulfur levels, whole bees and pollen were oven-dried and ground to pass a 40-mesh screen. An Orion specific ion probe was used for fluoride determinations, while sulfur determinations were performed using a Leco Induction Furnace. Previously, I reported difficulties in analyzing honey because of its extremely hygroscopic properties. Tong *et al.* (1975) detected sulfurs in honey, so I have continued to modify and improve methods for analyzing honey. I believe that I can now dry honey effectively by spraying or spreading it on a hot drum, a procedure used commercially to make dried honey (White, 1975). Honey analyses should be completed by autumn of 1978, but they were not complete as of the date of this report.

Trace Metals

Because I have found that honeybees are good indicators of the presence of fluoride pollutants, I decided to investigate the possibility of monitoring other elements emitted by the Colstrip power plants. Literature reviews (Bromenshenk, 1976; Debackere, 1972; Lillie, 1972) indicated that bees were known to accumulate arsenic and lead and that these substances have caused severe bee losses. In an on-going case brought by George Grant Ballantyne, Cloverdale Apiaries (Plaintiff vs. The Anaconda Company, District Court of the Fifth Judicial District of the State of Montana, 1976), the plaintiff alleges that his bees were killed by arsenic emitted by a copper smelter 72 km away. Data presented in the case clearly demonstrated that arsenic, lead, and cadmium do accumulate in or on the tissues of bees and can be detected by standard atomic absorption spectrophotometry procedures. The concentrations of these substances appeared to be related to the location of the bees with respect to an emission source.

I have not found any references to cadmium accumulation in bees or of its toxicity to bees, but this element is reported to be toxic to living organisms in all of its forms (Fulkerson and Goeller, 1973). As part of a pilot study, I chose to examine the accumulation of cadmium, lead, arsenic, and zinc because of data indicating the presence of these anthropogenic contaminants in both the ambient air and vegetation in the area surrounding Colstrip (Munshower and DePuit, 1976) and in stack emissions (Crecelius *et al.*, 1978).

I am analyzing bees for these substances using standard atomic absorption spectrophotometry procedures (Rowe, 1973) and a nitric-perchloric wet ash digestion procedure (Behan and Kinraide, 1970) which I have tested and which have proved to be successful. I intend to perform a series of standard addition calibrations of lead, cadmium, zinc, and arsenic to the bee matrix to provide quality assurance tests of the analytical procedures. Since I have tissue banks of frozen bees from 1974 to the present and since I can subsample bees collected for fluoride and sulfur analyses, no additional sampling effort is required for trace element determinations. This work is being carried out with the assistance of Dr. F. F. Munshower (Montana State University), who has the necessary equipment and qualified personnel to perform analyses for trace amounts of cadmium and arsenic; I am performing the zinc and lead analyses. Preliminary results will be presented in this report.

Radionuclides

In an attempt to determine the scavenging efficiency of honeybees for airborne particulates, I sent to Battelle Pacific Northwest Laboratories (BPNL) a sample of bees from an apiary located downwind from the Colstrip power plants near the EPA Hay Coulee air quality monitoring site.

Naturally-occurring radionuclides, such as the isotope Beryllium^7 which is formed in the stratosphere, fall upon the earth at a relatively constant and measurable rate. Other radioactive materials, such as fallout from the Chinese weapons testing, also reach the surface of the earth and the rates of fallout can be measured. By comparing the levels of these radioactive materials in or on bee tissues to those on static surfaces, such as leaves and some specifically-designed collection plates, one should be able to determine if bees accumulate radionuclides above the levels of stationary objects. This should provide an index to scavenging or accumulating efficiency. In addition, because different radionuclides have different particle sizes, one should be able to determine the size range of the particles the bees are likely to pick up during their foraging flights by expressing the levels of accumulation of these materials as ratios to some standard (in this case using Cesium^{137}) and taking into account known or measurable particle size. This procedure has been used by BPNL to determine deposition rates of marine aerosols on the sea surface (Crecelius *et al.*, 1978).

I became interested in the particulate scavenging efficiency of honeybees after computing the respiration rates of bees in flight using data published by Wigglesworth (1972). It did not seem conceivable that bees could accumulate by respiration alone as much fluoride as observed in bees from industrial areas. Whereas ingestion of fluoride-contaminated water or pollen appears to be a possible route of transport of fluoride into bees, this did not seem to be the route of passage of the increased fluoride seen in bees downwind from the Colstrip power plants (Bromenshenk, 1978a). Therefore, at least in some instances, particularly in Colstrip, I hypothesized that flying bees act as miniature impactors, picking up pollutants not only from surfaces of flowers or in water but also as they fly through contaminated atmospheres. At present, a preliminary feasibility test has been completed and the results are encouraging (included in the results section of this report). The majority of the radionuclide work will be performed during the 1978 field season.

RESULTS

The year 1977 was unusual as regards beekeeping. Drought conditions sharply reduced colony yield. According to the Montana Crop and Livestock Reporting Service, the average yield per colony dropped to 57 pounds from 112 pounds in 1976 and 94 pounds in 1975. Montana usually ranks first or second in the nation in yield per colony, but this year Montana dropped to fifth and, in terms of total honey production, fell from sixth to twelfth. At some locations, floral resources were so poor that colonies were in danger of starvation. This occurred near Colstrip for the first time since the initiation of our studies in 1974. Some apiary locations were not used at all during the 1977 growing season, and bees were taken out of non-productive (in terms of honey storage) apiaries by mid-summer. Thus, I did not have as many sample locations to work with as in previous years, and some locations sampled in June could not be sampled in September because the bees had been moved to other sites. I sampled 15 sites both in June and in September, so the sample grid was more or less complete. At some of the vacated sites, a few hives or at least one hive remained, so I had some data. The drought was an unusual occurrence, and I do not anticipate that it should be a problem during the 1978 growing season, since the snowfall up to April was unusually heavy. In spite of drought, the number of bee colonies in Montana has increased by 6 percent (96,000 colonies were registered in 1977).

Fluoride

As I have mentioned, when compared to baseline or pre-operational levels, fluoride concentrations in or on honeybees sampled in September of 1976 increased by as much as twofold at apiaries located as far as 15 km downwind (based on prevailing wind patterns) from the Colstrip power plants. At that time, one of the two power generators had been in operation 12 months; the other only three months. Neither power plant had operated continuously, and both had operated at approximately one-third capacity.

Baseline samples of soil, vegetation, and water indicated that naturally-occurring fluoride from geochemical sources in these areas occurred at low levels and did not display steep gradients of change. Thus, one would expect the concentration of fluoride in bees' food and water sources to be rather uniform.

Bees do not forage at random but display a preference for particular flowers and foraging areas (Doull, 1971). If an area becomes subjected to air pollution, one would then expect the concentrations of contaminants such as fluoride to be related to factors such as topography and meteorology. In other words, some areas would be more exposed to ambient pollution. Bees foraging in areas of greater impact should come in contact with more contaminants than bees foraging cleaner areas. Bees can forage up to 8 km from their hives, although they usually remain within 2 to 3 km (McGregor, 1976). Thus, since bees from different hives may forage in different directions, bees from one hive might bring back a relatively large quantity of pollutants while bees from another might bring back relatively little.

Because of this preferential foraging, I hypothesized that the variance of fluoride levels in bees in pollution-stressed areas should increase as the mean increased. Since my ability to monitor this was limited by a small sample size at any given location, I increased the number of individual hives being sampled from four in 1974, 1975, and 1976 to ten in 1977.

The number of beehives per apiary near Colstrip has varied from as few as 16 to as many as 50. Using Stein's two-stage sample test to determine the number of observations necessary for a mean with a confidence interval of a prescribed base, I evaluated the number of observations (n) based on 1975 and 1976 data and using the equation:

$$n = \frac{t_1{}^2 s^2}{d^2}$$

where t is the tabulated $t_{.05}$ value for the desired confidence interval and degrees of freedom, d the half-width of the desired confidence interval, and s^2 the sample variance. According to my computations, increasing the number of hives sampled from four to ten should decrease the confidence interval to 64 percent of the original width but would more than double collection time. Increasing sample size to 15 (the maximum size likely to be found at all sites) should decrease the confidence interval an additional 12 percent but would triple the collection time. Collection time is an important consideration because of the work necessary to obtain pollen, honey, bees, and water from individual hives plus a pooled sample of bees from all of the hives in each beeyard. One person usually could sample only two beeyards during an 11-hour day. Since I sample as many as 20 apiaries, ten hives per apiary would take a minumum of ten days, and 15 hives would take 15 days. This assumes good weather conditions. However, in autumn, rain and mud often interfere with sampling, and it often takes several extra days to get to all of the beeyards. I decided that increasing the sample size to ten was warranted but that the time needed to sample 15 would be prohibitive.

The results of fluoride analysis of the September, 1977, samples are compared to those for autumn of 1975 and 1976 in Figure 6.2. Increasing the sample size from four to ten hives narrowed the confidence limits to within the parameters estimated by Stein's two-way test. The data for fluoride in honeybees and statistical analyses are presented in Appendix 6.1.

I intend to examine the data for fluorides in honeybees by a variety of statistical techniques. However, at the time of preparation of this report, I had not completed the analyses of fluoride in bees collected during June and July. Also, preliminary tests suggested that the fluoride values for 1977 might not be normally distributed. Therefore, for this report, I have utilized non-parametric tests in lieu of parametric tests such as the anova analyses of variance which assumes normalcy of distributions (Sokal and Rohlf, 1969).

Fluoride concentrations in adult worker honeybees from individual hives at given locations were considered to be the basic group for comparisons of pre- and post-operational data. Using a Dec-20 computer, fluoride values for honeybee workers were ranked. First, all variates from all groups were pooled. The pooled variates were then sorted from lowest to highest (X_0, X_1, X_2 . . . X_n). Then, the pooled and sorted observations were ranked from low to high (R_1, R_2, R_3 . . . R_n) into the original groups and average ranks computed as:

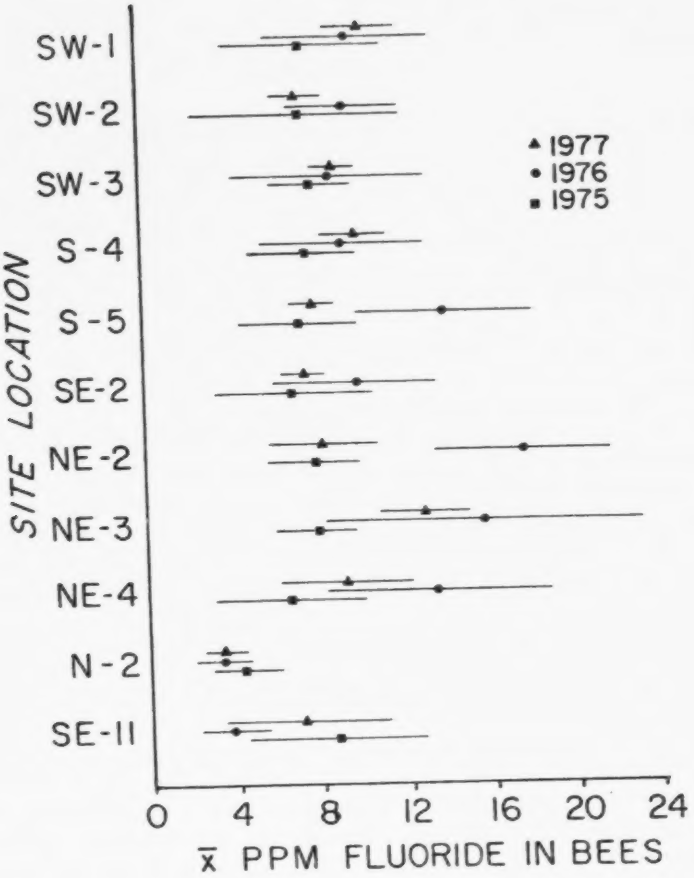


Figure 6.2. Mean fluoride and 95% confidence intervals. 1975 and 1976 values are from four independent samples; 1977 values are from ten independent samples, except for SE-11 and NE-2, which are each from three samples.

$$n_i$$

$$\frac{(\Sigma R)_i}{n}$$

This was done for the autumn sample collections for all sites and all years combined (1975-77), for the 14 principal sites by year (1975 and 1977), and for six sites downwind from Colstrip in terms of prevailing winds and within 20 km of Colstrip for each year (1975 and 1976).

The tests employed include the Kruskal-Wallis and the Wilcoxon two-sample tests. The Kruskal-Wallis tests the null hypothesis that all the populations have the same distribution of fluoride concentrations. The alternative hypothesis is that some populations provide higher distribution values than others. A significant value for the Kruskal-Wallis statistic causes us to reject the former and to accept the latter. The results are presented in Table 6.1.

TABLE 6.1. KRUSKAL-WALLIS TEST OF FLUORIDE IN ADULT WORKER HONEYBEES

Site	Year	Kruskal-Wallis Statistic, H	df	Critical χ^2	P
All Sites (n = 48)	1975-77	151.50	47	63.98	$P \leq 0.001$
Principal Sites (n = 14)	1975	21.78	13	22.36	n.s.
	1977	54.74	13	22.36	$P \leq 0.001$
S-4, S-5, SE-2, NE-2, NE-3, NE-4 (downwind from Colstrip)	1975	2.52	5	11.07	n.s.
	1976	12.14	5	11.07	$P \leq 0.05$
	1977	18.77	5	11.07	$P \leq 0.005$

Based on these results, I accepted the null hypothesis for 1975. Pre-operational fluoride distributions were the same at all locations. For 1976 and 1977 (post-operational), honeybees displayed a higher distribution of fluoride concentrations than during 1975.

The Wilcoxon two-sample test was used to determine whether there was a significant difference in the distributions of the levels of fluoride in or on bees at a given location (apiary) from one year to the next. In other words, I used it to test if the distribution of post-operational concentrations of fluoride in 1977 was greater than those of the pre-operational concentrations or those of the 1976 post-concentrational period. If X designates 1975 values, Y 1976 values, Z 1977 values, and E expected, then the null hypotheses may be stated as follows:

A (one-tailed test)	H_0: $E(Y) \leq E(X)$ H_1: $E(Y) > E(X)$
B (one-tailed test)	H_0: $E(Z) \geq E(Y)$ H_1: $E(Z) < E(Y)$

These comparisons were made using the average rank data graphed in Figure 6.3 for those sites for which change in average rank appeared to have occurred. The Wilcoxon test results are presented in Table 6.2.

TABLE 6.2. WILCOXON TWO-SAMPLE TEST

Site	Year	Wilcoxon Statistic, T	Critical Value	P
NE-3	1975-77	35.00	27	$P \leq 0.001$
S-4	1975-77	30.00	30	$P \leq 0.05$
NE-2	1976-77	39.00	38	$P \leq 0.005$
NE-4	1976-77	34.00	33	$P \leq 0.05$
S-5	1976-77	39.00	38	$P \leq 0.005$
SW-2	1976-77	35.00	35	$P \leq 0.025$

Based on the Wilcoxon two-sample test, I concluded that the distribution of fluoride levels in honeybees at two sites near Colstrip, NE-3 and S-4, was significantly greater for 1977 than for 1975. However, at four sites near Colstrip, NE-2, NE-4, S-5, and SW-2, the distribution of fluoride in honeybees was significantly smaller for 1977 than for 1976.

Based on the Kruskal-Wallis and Wilcoxon tests and the average rank data depicted in Figure 6.3, the distribution of fluoride concentrations in honeybees in 1977 tended to be equal to or higher than that of baseline levels at sites within 20 km of Colstrip and less than that of baseline levels at sites more than 40 km away. As compared to 1976, the distribution of 1977 fluoride values tended to be equal to or lower than those of the previous year. If changes in fluorides in honeybees are caused by emissions from the Colstrip power plants, then one would expect these changes to correspond to wind patterns which would carry the plume to the areas at which higher values occurred. Figure 6.4 summarizes 1976 and 1977 wind data for May through September, *i.e.*, the period the honeybees were present at sites within 20 km of Colstrip. Wind data was supplied by the Montana State Department of Health

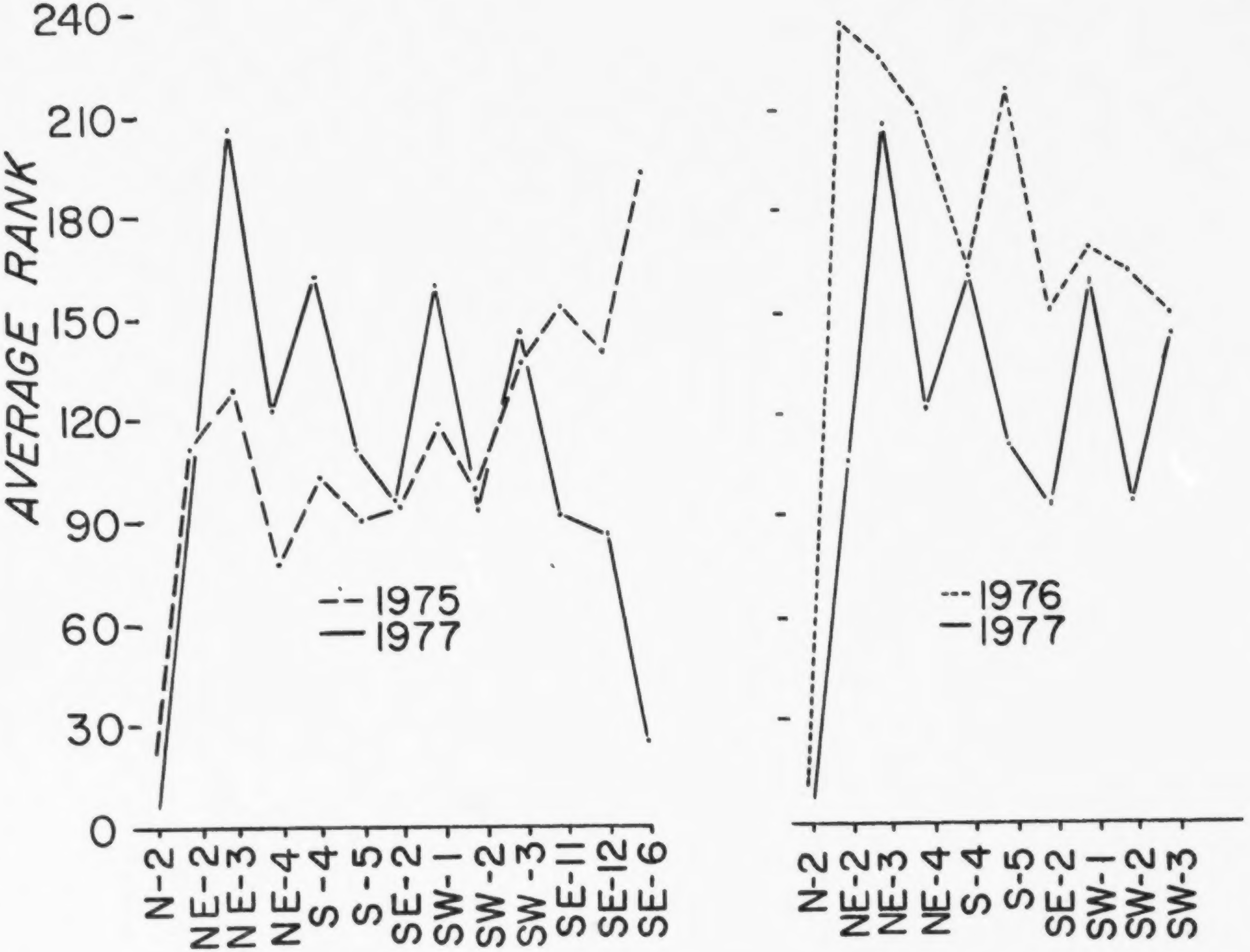


Figure 6.3. Average rank of fluorides in honeybees from southeastern Montana used in the application of the Kruskal-Wallis test.

and was measured atop a 98.3 m tower astride a 65.6 m hill, 1,400 m north-northeast of the Colstrip power plants.

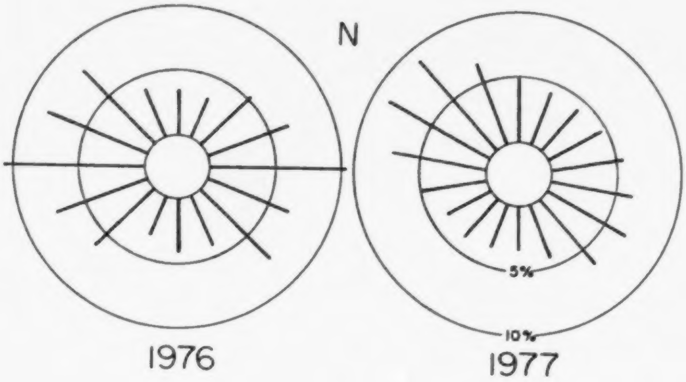


Figure 6.4. Wind roses for Colstrip, Montana, May through September, 1977; measured at 164 m above the surrounding terrain, 1,400 m north-northeast of the power plants.

As I reported in the Third Interim Report to EPA (Bromenshenk, 1978a), increases in mean fluorides in bees in 1976 compared to 1975 occurred at those sites downwind and as far as 20 km from Colstrip. In 1977, the two apiaries demonstrating significantly greater distributions of fluoride over those of the 1975 baseline also occurred in quadrants receiving frequent winds.

It should be noted that although the most prevalent winds during the 1977 growing season came from the same quadrants as in 1976, the wind directions were more variable in 1977 than in 1976.

The mean fluoride content of apiary water supplies was 0.34 ppm ($S_{\overline{x}}$ = .04) in autumn of 1977. The fluoride content of these same water supplies was 0.46 ppm ($S_{\overline{x}}$ = .06) in autumn of 1976. The correlation coefficient for fluoride in water for September of 1976 and 1977 was significant (r = .81, df = 10, $P \leq 0.01$ by Snedecor's tabular values for the t test of r), indicating a high degree of association. Mean fluoride in honeybees sampled in 1977 did not correlate with fluoride in water (r = 0.14, df = 11, not significant by Snedecor's tabular values). Thus, mean fluoride in honeybees did not appear to be dependent on changes of fluoride levels in water.

Trace Metals

The results of our preliminary determinations of arsenic and zinc concentrations in honeybees are presented in Table 6.3. Too few arsenic analyses were completed at the time of this report for any meaningful pre- and post-operational comparisons. However, it is readily apparent that honeybees from southeastern Montana have accumulated much less arsenic than bees from an

TABLE 6.3. ARSENIC AND ZINC BIOASSAYS OF HONEYBEES FROM COLSTRIP, MONTANA

Date	Sample	Ppm	Average Bee Weight (dry)	µg Arsenic/ Average Bee
ARSENIC (Bioassays by D. Neuman and F. Munshower, Montana State University)				
	Colstrip			
Autumn 1974	S-5	0.81	0.029	0.024
	S-1	0.69	0.030	0.021
Autumn 1976	N-1	0.38	0.034	0.013
	N-1	0.62	0.030	0.018
	S-1	0.40	0.038	0.015
	S-5	0.19	0.043	0.008
	S-1	0.67	0.026	0.017
	S-5	0.47	0.028	0.013
	Columbia Falls			
Spring 1975	003	0.57	0.028	0.016
	004	2.03	0.026	0.054
	Butte			
Spring 1976	005	3.06	0.035	0.106
ZINC (Bioassays by Environmental Studies Laboratory, University of Montana)				
	Colstrip			µg Zinc/ Average Bee
Autumn 1974	NE-3	87	0.036	3.13
	NE-4	68	0.037	2.52
	SE-2	80	0.029	2.34
	SW-1	82	0.030	2.46
	S-5	93	0.030	2.79
	E-1	112	0.028	3.14
Spring 1977	NE-3	97	0.026	2.52
	NE-4	101	0.030	3.03
	NE-10	99	0.027	2.67
	SE-6	97	0.031	3.01
	SE-12	95	0.033	3.15
	S-1	90	0.028	2.52
	S-4	137	0.033	4.52
	S-5	157	0.029	4.55
	SW-1	136	0.029	3.94
	SW-2	126	0.028	3.53
	SW-3	123	0.025	3.08
	N-1	124	0.027	3.35

apiary near Butte or bees from one of the two apiaries sampled near Columbia Falls. In the Butte area, a copper smelter is a known source of arsenic pollutants. In the Columbia Falls area, the apiary at which over 2 ppm arsenic occurred in the bees is located downwind from an aluminum reduction facility; bees from this beeyard also had approximately 80 ppm fluoride, as compared to about 25 ppm fluoride and 0.57 ppm arsenic at an apiary upwind from the aluminum plant.

Figure 6.5 depicts average arsenic levels in the tissues of worker honeybees from different areas of Montana and includes data submitted by the plaintiff in the case against the Anaconda Company mentioned earlier in this report. Figure 6.5 demonstrates that honeybees from polluted areas may contain more than 20 times as much arsenic as honeybees from Colstrip.

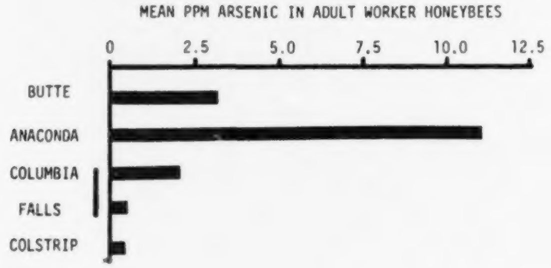


Figure 6.5. Arsenic in honeybees from clean and polluted areas of Montana.

The levels of zinc in honeybees from sites near Colstrip in autumn of 1974 averaged 87 ppm, while zinc in bees in the spring of 1977 averaged 115 ppm. The higher level in the spring of 1977 could reflect a seasonal variability. I am currently analyzing samples obtained in September, 1977, to determine if zinc concentrations remained at this level. Snowfall at some locations near Rosebud Creek in 1976-77 had relatively high concentrations of zinc (K. Beisinger, EPA-Duluth, personal communication). Rosebud Creek is where most of the apiaries near Colstrip are located.

Radionuclides

The results of a preliminary test for radionuclides in bees obtained in September of 1977 from site S-5 are presented in Table 6.4. Potassium^{40} is a normal constituent of biological tissues; Be^{7} is formed in the stratosphere. It was assumed that the other radionuclides were released by a Chinese atmospheric weapons test conducted almost a year before the sample period.

Radionuclides can be detected in or on the bodies of honeybees, and these materials do occur in different quantities. More extensive tests will be conducted during the 1978 growing season. The results and data interpretation should be available for inclusion in the Fifth Interim Report.

TABLE 6.4. RADIONUCLIDES ON OR IN HONEYBEES, SITE S-5, COLSTRIP, MONTANA, SEPTEMBER, 1977

Element	Disintegrations/Minute/100 gm (wet weight)	Enrichment Factor (ratio to Cs^{137})
Be^{7}	44.5	25.30
Ce^{144}	22.1	12.50
Ce^{141}	3.38	1.92
Cs^{137}	1.76	1.00
K^{40}	453.0	257.00
Nb^{95}	31.6	18.00
Zr^{95}	14.5	8.24

DISCUSSION

The purpose of all of the studies discussed in this report has been to determine the bioenvironmental impacts of coal-fired power plants and to generate methods to predict the bioenvironmental effects of air pollution from these facilities before damage is sustained. The ultimate objective is to provide planners and decision makers with a means of assessing and mitigating the ecological and related socio-economic impacts of siting coal energy conversion plants in the Northern Great Plains. Because this project is concerned with the impacts of emissions from the two 350 MW power plants at Colstrip, Montana, a brief description of these emissions is needed before our research can be discussed.

We began our investigations in August, 1974. The first Colstrip power plant (Unit 1) went on-line in September, 1975. Unit 2 began operations in June, 1976. Neither generating plant averaged better than 50 percent capacity, except for brief periods, until March, 1977 (Section 5).

The effects of emissions from these power plants on air quality have been monitored at a variety of sites by EPA-Corvallis, Battelle Pacific Northwest Laboratories, State of Montana, National Oceanic and Atmospheric Administration, and the Montana Power Company. Unfortunately, the available air quality data provides only a very superficial profile of the plume dispersion parameters (O'Toole *et al.*, 1978). However, the plume has been detected several times a week at the monitoring station at Hay Coulee, approximately 11 km southeast of Colstrip. This indicates that fumigation will probably occur at other areas downwind of Colstrip. The Hay Coulee monitor is situated between the apiaries at S-5 and SE-1, about 10 km and 15 km, respectively, from Colstrip.

Crecelius *et al.* (1978) reported that particles emitted by the stack of Unit 2 in March, 1977, were extremely small (80 percent less than 0.5 µm in diameter) and were enriched in fluoride, arsenic, zinc, lead, mercury, vanadium, copper, selenium, and antimony. These authors reported that because 69 percent (by weight) of the particles from the stack were less than 0.3 µm in diameter, the flyash from the power plant could remain airborne for days or weeks and be transported 100 to 1,000 km unless removed by rain, washout, or agglomeration of the particles.

Crecelius' *et al.* data on concentrations of elements in stack flyash from Unit 2 included 39 elements. The stack concentrations of those elements that I examined in honeybees are as follows:

Element	ppm	± SD
F	2,130	400
As	221	20
Zn	221	79
Pb	252	25

In addition, Crecelius *et al.* suspected sulfur dioxide and several elements (Hg, F, As, Se) to be present in significant concentrations as a vapor in the stack gases.

These researchers also sampled the plume using a DC-3 airplane during February, 1977, and a helicopter during August, 1977. The results of the samplers indicate that the plume could be followed 50 km downwind, that particle size increased from primarily submicron (0.01 to 0.1 µm) near the stack to one micron particles beyond 8 km, while particle numbers per cm^3 decreased, and that plume width and thickness increased with distance from the stack. Using the helicopter, the plume could be traced down to 10 m above the ground, 5 km southeast of Colstrip. They concluded that their plume sampling supported our data showing rising concentrations of sulfur and fluoride in vegetation (Section 5). I believe that their data also supports our observations of increasing fluoride levels in mice (Gordon *et al.*, 1978) and in honeybees.

The 1976 and 1977 wind rose data, obtained from a 306 m tower on a hill just north of the Colstrip power plants, indicates that winds frequently blow towards the southeast and the northwest. Winds also come from the southeast, but there were no apiaries located northwest of Colstrip because there were no suitable forage locations for honeybees in that area.

Although I do not have air quality data for 1977 for any of the apiaries northeast of Colstrip, I have frequently observed the plume from the power plants descending to the ground and following drainage routes to the northeast in a direction that presumably would carry it directly over the NE-2 and NE-4 apiaries. Thus, it would appear that those beeyards at which I observed post-operational increases in mean fluoride content or higher distribution functions of fluorides in bees were subjected to plume strikes. But I lack data concerning air quality at these locations. Because of this, I intend to place sulfation and formate plates near these apiaries during the 1978 growing season.

Why did the distribution of fluoride levels in honeybees at four sites near Colstrip decrease in September, 1977, from that of September, 1976? A similar change was observed in the concentrations of fluorides in vegetation at some of the sites near Colstrip. I have hypothesized several explanations for this response:

(1) The winds were more variable in 1977 than in 1976 so that pollution episodes may have been more frequent at downwind beeyards in 1976.

(2) Because of the "drought," honeybee production was about one-half that of either of the previous two years (G. Simpson, beekeeper, personal communication). During periods of poor forage availability, honeybees reduce flight activity and foraging, sending out mainly scout bees (Doull, 1971), so that the amount of time during which large numbers of bees might encounter fumigations by the plume would be reduced. This would be particularly true if bees were picking up contaminants primarily during flight rather than from material which had settled on vegetation or in water.

(3) Both mean fluoride and distribution functions of fluoride levels in bees from apiaries 40 to 80 km from Colstrip tended to be lower than those of 1975 (baseline) levels. At one location, the levels were significantly lower. This would support the hypothesis that decreased flight and foraging could result in decreased gathering of substances such as fluoride. An alternate explanation could be that the 1977 data reflect some type of seasonal difference, but my 1975 and 1976 studies did not reveal any significant seasonal variability in fluorides in honeybees (Bromenshenk, 1978a). Whatever the explanation, the mean fluoride and the distribution of fluoride levels in honeybees sampled in September of 1977 tended to be somewhat lower than expected throughout the entire region.

(4) Although the Colstrip power plants operated relatively continuously in 1977 and at a greater capacity than in 1976 (Montana Power Company data, see Section 5), during mid-August through September, they operated at a level only 16 percent greater than that of 1976, based on coal consumption. Presumably, they emitted proportionally greater amounts of fluorides in 1977. A worker bee lives about five to six weeks during the peak activity periods of summer and early fall. They do not begin to fly until approximately 20 days old, so the time during which they would be likely to be in direct contact with air pollutants would be two or three weeks. Thus, the operational history of the power plants during the month preceeding the sample period rather than on an annual basis is probably more important in relation to levels in honeybees, unless long-term accumulation in food and water supplies is involved.

(5) There is the possibility that less fluoride was emitted at Colstrip in 1977 than in 1976 resulting from changes such as differences in the fluoride content of the coal utilized. Also, it is possible that some of the fluoride detected in the honeybees may have been released by activities such as blasting in the coal strip mine at Colstrip, although one would not expect that much, if any, fluoride released in the mines would be carried over intervening ridges to apiary locations several kilometers distant.

Preliminary data concerning trace elements clearly demonstrate that honeybees accumulate a variety of elements in addition to fluoride and support my belief that honeybees can be used as efficient detectors of a variety of pollutants.

The radionuclide studies are based on techniques developed by Battelle Pacific Northwest Laboratories to determine deposition rates of continental aerosols on desert plants and marine aerosols on the sea surface. The initial results indicate that the method should be applicable to the Colstrip plants, assuming that the stack particulates act in a manner similar to ambient aerosols (Crecelius *et al.*, 1978). This procedure should provide data on the scavenging efficiency of honeybees as compared to deposition of aerosols on vegetation and soil. Data for all three should facilitate determinations of the rate of particulate deposition in regions downwind from Colstrip.

CONCLUSIONS

I have conducted two years of post-operational investigations into the bioenvironmental impacts of the coal-fired power plants at Colstrip, using indicator species of insects as early warning systems of damage and continuous monitors of atmospheric pollutants. Whereas this section concentrates on the use of honeybees as accumulators of anthropogenic contaminants and susceptibility of pollinators to poisoning from these substances, I also have been monitoring insect damage to ponderosa pines (Section 5) and the impacts of exposures to long-term low-level concentrations of sulfur dioxide on ground-dwelling, saprophagous and predatory beetles at the EPA ZAPS (Section 18).

Honeybees sampled in autumn of 1977 continued to demonstrate significant post-operational changes in terms of the distribution of fluoride levels in their body tissues at apiaries downwind and within 25 km of the power plants. Mean fluoride levels in bees declined somewhat compared to 1976, and fluoride levels returned to the 1975 baseline levels at some but not all locations. At distant sites (40 to 80 km), the September, 1977, fluoride levels were as low or lower than baseline levels. In one instance, the fluoride levels were significantly lower. This suggests a regional depression of fluoride concentrations in honeybees which was offset near Colstrip by emissions from the power plants. An unusually dry growing season almost halved production per hive and presumably would have decreased flight and foraging activities by honeybees. This may have decreased the amount of contact honeybees had with fluoride contaminants in the area.

The prevalent winds at Colstrip from May through September of 1977 continued to blow towards those apiaries located southeast and northeast of the power plants. However, in 1977, the winds were more variable and blew less frequently to the southeast and northeast than in 1976. Thus, there was a good possibility that plume strikes occurred less often at the downwind apiary sites in 1977.

Only one power plant was in operation throughout the 1976 growing season. The other went on-line in mid-summer of that year. During the 1977 growing season, both plants were on-line and had increased their output. Presumably, pollutants being emitted also increased proportionally. However, based on coal consumption data provided by the utility companies, total power plant operation during the month prior to our September, 1977, sampling of apiaries was only about 16 percent greater than in the same period in 1976.

Data drawn from a variety of ambient air quality studies in the Colstrip region for 1977 provided evidence that the plume from the power plant frequently hits the ground 5 to 12 km southeast of Colstrip. This supports my observations of increased fluoride distribution functions in honeybees in this area. Presumably, the plume also may strike the ground at similar distances northeast of the stacks, but there have been no attempts to monitor it there.

I am convinced that honeybees are excellent indicators for fluoride impacts, and I have initiated studies of other trace elements in honeybees. At present, I have not completed enough analyses for meaningful pre- and post-operational evaluations of these contaminants at Colstrip. However, I have found that arsenic levels in bees from apiaries near recognized sources such as a copper smelter were as much as 20 times that in Colstrip bees. Zinc in honeybees near Colstrip in the spring of 1977 was 1.3 times higher than levels in the fall of 1975, although this could reflect seasonal variability. However, zinc is one of the elements emitted by the Colstrip power plants.

Several radionuclides have been detected in or on the tissues of worker honeybees. Included were radionuclides associated with the Chinese atmospheric weapons tests, almost a year prior to the sample period. The purpose of the radionuclide studies, which have just begun, is to provide data concerning the scavenging efficiency of honeybees as related to particle size of pollutants.

In view of the contaminants that honeybees accumulate and the degree of magnification of these materials in bee systems compared to that in their environs, the effects of these substances on the bees themselves and on pollination systems should be more intensely studied. Thus far, the build-up of toxic materials in bees near Colstrip has not reached that reported to be injurious to bees. However, neither power plant has been in continuous or full capacity operation, and two years of post-operational studies is a relatively short period considering that contaminants may slowly accumulate in the surrounding ecosystems. Thus, I still do not have enough information to predict long-term consequences of the power plant emissions to honeybees. I believe that it is important that such information (for prediction) be gathered since the Northern Great Plains is an area of burgeoning coal development and power plant construction. This becomes of utmost importance to beekeepers and growers, not only of the Great Plains but elsewhere, because thousands of colonies of honeybees are transported in autumn to the West Coast and the Southwest where they are used to pollinate almond groves, orchards, and vegetable crops. Then, in the spring, they are returned to the Great Plains where they produce one of the largest honey and wax crops in the nation.

REFERENCES

Behan, M.J., and T. Kinraide. 1970. Rapid Wet Ash Digestion of Coniferous Foliage for Analysis of Potassium, Phosphorus, Calcium, and Magnesium. Bulletin 39. Montana Forest and Conservation Experiment Station, School of Forestry, University of Montana, Missoula, Montana. 7 pp.

Bromenshenk, J.J. 1976. Investigations of the Effects of Coal-Fired Power Plant Emissions Upon Insects, Report of Progress. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Second Interim Report, Colstrip, Montana, June, 1975, R.A. Lewis, N.R. Glass, and A.S. Lefohn, eds. EPA-600/3-76-013, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 112-129 and 286-312.

________. 1978a. Investigations of the Impact of Coal-Fired Power Plant Emissions Upon Insects. I. Entomological Studies in the Vicinity of Colstrip, Montana. II. Entomological Studies at the Zonal Air Pollution System. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Third Interim Report, Colstrip, Montana, December, 1977, E.M. Preston and R.A. Lewis, eds. EPA-600/3-78-021, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 146-312 and 473-507.

________. 1978b. Yet Another Job for Busy Bees. The Sciences, 18(6):12-15.

________, and C.C. Gordon. 1978. Terrestrial Insects Sense Air Pollutants. In: Proceedings of the Fourth Joint Conference on Sensing of Environmental Pollutants, November 6-11, 1977, New Orleans, Louisiana. American Chemical Society, Washington, D.C. pp. 66-70.

Crane, E., ed. 1975. Honey, A Comprehensive Survey. Crane, Russack, and Company, Inc., New York. 608 pp.

Crecelius, E.A., L.A. Rancitelli, and S. Garcia. 1978. Power Plant Emissions and Air Quality. In: Potential for Gaseous and Heavy Metal Contamination from Energy Extraction Processes in the Northern Great Plains and the Consequent Uptake and Turnover in Range Ecosystems. ERDA Annual Report, Activity RX-02-03. Ames Laboratory, Iowa State University, Ames, Iowa. pp. 9-33.

Debackere, M. 1972. Industriele Luchtvervuiling en Bijenteelt (Industrial Pollution and Apiculture). Vlaams Imkersblad., 2(6):145-155.

Doull, K.M. 1971. An Analysis of Bee Behavior as it Relates to Pollination. Am. Bee J., 111(7):266,273; (8):302-303; (9):340-341.

Fulkerson, W., and H.E. Goeller, eds. 1973. Cadmium, the Dissipated Element. ORNL NSF-EP-21. Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Gordon, C.C., P.C. Tourangeau, and P.M. Rice. 1978. Potential for Gaseous Contamination from Energy Extraction Processes in the Northern Great Plains. In: Potential for Gaseous and Heavy Metal Contamination from Energy Extraction Processes in the Northern Great Plains and the Consequent Uptake and Turnover in Range Ecosystems. ERDA Annual Report, Activity RX-02-03. Ames Laboratory, Iowa State University, Ames, Iowa. pp. 53-137.

Hillmann, R.C. 1972. Biological Effects of Air Pollution on Insects, Emphasizing the Reactions of the Honey Bee (*Apis mellifera* L.) to Sulfur Dioxide. Ph.D. Thesis, The Pennsylvania State University, University Park, Pennsylvania. 159 pp.

Lillie, R.J. 1972. Air Pollutants Affecting the Performance of Domestic Animals, A Literature Review. Agriculture Handbook No. 380. USDA Agricultural Research Service, Washington, D.C. 109 pp.

McGregor, S.E. 1976. Insect Pollination of Cultivated Crop Plants. Agriculture Handbook No. 496. USDA Agricultural Research Service, Washington, D.C. 411 pp.

Munshower, F.F., and E.J. DePuit. 1976. The Effects of Stack Emissions on the Range Resource in the Vicinity of Colstrip, Montana. Research Report 98. Montana Agricultural Experiment Station, Montana State University, Bozeman, Montana. 111 pp.

O'Toole, J.J., C.C. Gordon, L.A. Rancitelli, E.A. Crecelius, S. Garcia, F.F. Munshower, E.J. DePuit, P.C. Tourangeau, and P.M. Rice. 1978. Potential for Gaseous and Heavy Metal Contamination from Energy Extraction Processes in the Northern Great Plains and the Consequent Uptake and Turnover in Range Ecosystems. ERDA Annual Report, Activity RX-02-03. Ames Laboratory, Iowa State University, Ames, Iowa. pp. 1-8.

Rowe, C.J. 1973. Food Analysis by Atomic Absorption Spectroscopy. Varian Techtron Pty., Ltd., Palo Alto, California. 47 pp.

Siewniak, M. 1971. Uszkadzanie Sosny Pospolitej (*Pinus sylvestris*) Przez Czerwca Korowinowca (*Matsucoccus pini* Green, 1925; Margarodidae, Coccoidea). Sylwan, 115(12):35-41.

Sokal, R.R., and F.J. Rohlf. 1969. Biometry. The Principles and Practice of Statistics in Biological Research. W.H. Freeman & Company, San Francisco, California. 776 pp.

Steche, W. 1975. Industrial Development and Its Effects on Beekeeping. Apiacata, 10(3):119-124.

Tong, S.C., R.A. Morse, C.A. Bache, and D.J. Lisk. 1975. Elemental Analysis of Honey as an Indicator of Pollution. Arch. Environ. Health, 30:329-332.

Toshkov, A.S., M.M. Shabanov, and N.I. Ibrishimov. 1973. Attempts to Use Bees to Prove Impurities in the Environment. Comptes rendus de l'Academie bulgare des Sciences, 27(5):699-702.

White, J.W., Jr. 1975. Composition of Honey and Physical Characteristics of Honey. A Comprehensive Survey, E. Crane, ed. Crane, Russack, and Company, Inc., New York. pp. 157-239.

Wigglesworth, V.B. 1972. The Principles of Insect Physiology. John Wiley and Sons, Inc., New York. 827 pp.

APPENDIX TABLE 6.1. MEAN FLUORIDE IN ADULT HONEYBEES (SEPTEMBER, 1977)

	Hive Number/Ppm Fluoride													
Site	1	2	3	4	5	6	7	8	9	10	$\bar{x}$	SD	SE	Combined Sample*
NE-2	16.6	11.8	8.7	8.6	7.9	7.6	5.6	5.6	5.4	4.8	8.3	3.6	1.14	12.0
NE-3	17.3	16.6	15.2	14.8	13.2	11.0	10.9	10.3	9.2	-	13.2	3.0	0.98	13.8
NE-4	19.4	11.0	10.4	10.1	10.1	8.4	6.2	6.2	5.5	4.8	9.2	4.2	1.34	13.2
NE-10‡	155.5	38.1	54.1	47.2	15.3	8.2	5.3	4.0	3.8	3.6	33.5	47.1	14.88	--
SE-11	9.0	7.0	5.8	--	--	--	--	--	--	--	7.3	1.6	0.93	7.8
SE-6	6.5	5.8	5.8	5.4	4.7	4.6	4.5	3.8	3.5	3.2	4.8	1.1	0.34	6.3
SE-12	13.4	10.6	7.5	7.2	6.5	6.4	6.1	5.7	5.7	5.2	7.4	2.6	0.82	6.7
S-4	13.7	11.6	11.1	10.4	10.2	9.0	8.4	7.7	7.3	--	9.9	2.1	0.68	10.8
S-5	11.1	9.2	8.8	8.0	7.9	7.7	7.0	6.6	6.4	6.3	7.9	1.5	0.47	8.0
SE-2	11.1	8.6	7.4	7.2	7.2	7.2	6.6	6.4	6.3	5.9	7.4	1.5	0.48	7.9
SW-1	11.7	13.5	11.7	11.1	10.7	10.5	8.3	8.2	6.9	6.6	9.9	2.3	0.72	7.7
SW-2	10.5	9.2	8.2	7.7	7.2	7.0	7.0	6.4	5.6	5.0	7.4	1.6	0.52	7.0
SW-3	11.0	10.8	10.5	10.3	9.2	8.8	8.3	7.9	7.5	6.6	9.1	1.5	0.48	8.1

*Each value represents a combined sample from 15-30 hives.
‡Hives 4-10 located farther from water source containing fluoride.

SINGLE HIVE, SUBSAMPLED:

Site	Ppm Fluoride			$\bar{x}$	SD	SE
N-2	3.7	3.7	3.0	3.5	0.4	0.23
SE-1	8.4	6.9	6.5	7.3	1.0	0.58

SECTION 7

TRENDS IN BIRD POPULATIONS IN THE VICINITY OF COLSTRIP

E. M. Preston and S. K. Thompson

ABSTRACT

Trends in bird populations have been monitored for three consecutive years in the vicinity of two 350 megawatt coal-fired power plants in Colstrip, MT. A census route patterned after the North American breeding Bird Survey was laid out along an anticipated gradient of pollution impact. Census data collected prior to operation of the power plants (1975) is compared with those collected after operation began (1976-77). Eighty-one species have been observed thus far. On the average, Western Meadowlarks accounted for 38% of the birds observed. Twenty-one additional species each contributed one percent or more to total abundance. Since 1975, the bird community has become increasingly dominated by Meadowlarks while the relative abundances of raptors, blackbirds and Lark Buntings have decreased. Species evenness (J') has decreased steadily since 1975 while the average number of species observed per site has increased. Much of the local variation in bird species diversity is strongly correlated with habitat factors. The presence of human structures, the proportion of area cultivated, the proportion of pine-juniper forest, the presence of riparian habitat and the distance from Colstrip were positively associated with species diversity. The presence of strip mining and the proportion of native grass coverage were negatively correlated with diversity. The ranges of bird species tend to be restricted by environmental factors within the study area. Consequently, the spatial distributions of species along the census route are often bimodal with one mode over zero counts and the other mode representing the most common count of the species. The negative binomial cannot adequately describe such a bimodal situation. However, it can be modified to allow for added zeros. The spatial distributions of Western Meadowlarks and Lark Buntings can be usefully described by the negative binomial with added zeros. The three parameters of the distribution are intuitively interpretable and may ultimately prove to be useful indicators of changes in patterns of space utilization.

INTRODUCTION

With the projected expansion of utilization of coal from the West, the Northern Great Plains are likely to be exposed to air pollution from coal-fired power plants. Chronic exposure to air pollution may cause changes in the native grassland ecosystem structure and dynamics. Birds are the most visible vertebrates of the grasslands from spring to late fall. They are potentially important indicator species because they are long-lived secondary consumers. They are unlikely to show acute effects of pollutants but may suddenly and belatedly exhibit severe damage when pollutants have accumulated to toxic levels in organ systems (Stickel, 1975). Their long lives enhance potential for accumulation of pollutants. We summarize here trends observed in bird community structure in the vicinity of two 350 megawatt coal-fired power plants in Colstrip, Montana.

MATERIALS AND METHODS

Census data were used to infer species dispersion and relative abundance patterns. The census was patterned after the widely employed North American Breeding Bird Survey (Robbins and Van Velzen, 1970). The observer starts one half hour before local sunrise and makes 60 three-minute stops at 0.8 km intervals along a predetermined route (see Lewis *et al.* 1976). At each stop, the number of birds of each species seen in a 400 m radius and heard, regardless of distance, is recorded. Since bird species have differential detectability, the census data provide no information on absolute abundance (Emlen, 1971). However, it should be possible to detect changes in species relative abundance patterns by comparing data obtained during a baseline period with those obtained using the same census technique at other times.

The census route was selected to provide sampling stations at varying distances from the Colstrip power plants along an anticipated gradient of pollution impact. Stops ranged from 1.2 km to 18.2 km from the Colstrip power plants. The stations cover a wide range of habitats including open grassland, streams, rolling hills with ponderosa pine and juniper coverage, cliffs and habitats affected by a wide range of human impacts including urban activities, railroad right-of-way, farming, ranching, and mining.

The 1975 breeding season is considered the baseline period since the power plants were not operational at that time. The route was censused on nine dates between May 6 and September 11, 1975. Generating Unit 1 began operation in September 1975 and Unit 2 in June 1976. The route was censused 21 times between April 21 and September 23, 1976, and 12 times between April 19, and September 9, 1977.

We hope to be able to relate structural descriptive parameters, such as species diversity, species richness, species evenness and degree of dispersion to changes in functional relationships between species and their environments. Species diversity was measured by the Shannon-Weaver function $(H' = -\sum_{n=1}^{s} P_i \log_2 P_i$ (Shannon and Weaver, 1949). Species richness (S) was measured by the number of species present, and evenness in species frequencies was measured by $J' = H'/\log_2 S$ (Pielou, 1969, 1975). To inspect overall temporal trends in these parameters, total H', total S and total J' were calculated from lumped census data for all sites on each sampling date. Plots of H' vs. number of sites included indicated that H' stabilized after approximately 40 sites had been included. Spatial and site specific trends were inspected by calculating total across season H', S, and J' from lumped data from representative sampling dates for each site. In between year comparisons care was taken to lump similar sample numbers and dates in calculating total across season values.

Habitat types at the census sites were quantified from aerial photographs taken from small aircraft 2500 feet above the ground. Native grassland, pine-juniper forest, and grain agriculture are the dominant habitat types. The percent area covered by these types within 400 m of census sites was estimated to the nearest 20%. Riparian habitat, strip mining operations, and human structures were classified as present or absent.

RESULTS AND DISCUSSION

The survey data provide information on spatial and temporal patterns in the distribution and abundance of species. The data can be used to monitor changes in dispersion and species composition associated with changes in the environment.

Species Composition

Both qualitative and quantitive changes in species composition can occur. Qualitative changes involve changes in species makeup resulting from the loss of species, the colonization of new species, or both. Such changes usually follow gradual quantitive changes in species relative abundance. They can be detected by inspection of a list of species occurrences during successive years. One year time intervals are appropriate because there are many seasonal changes in species occurrence not associated with long term trends. Annual species lists do not reflect most of these confounding changes. By studying contrasts between the life history strategies of those species favored versus those disfavored by pollution impact, insights into the wide ranging ecological effects being manifested at the systems level may be achieved. If generalist species tend to be replacing or increasing at the expense of specialists, retrograde succession may be indicated. If a few specialist species with similar adaptations seem to be favored over others, a directional shift in environmental selective pressures is indicated. Inspection of unique adaptations in the newly favored species may permit the new selective pressures to be deduced.

Table 7.1 summarizes the species composition observed during the breeding seasons of 1975, 1976, and 1977 in the Colstrip vicinity. Eighty-one species have been observed thus far. An average of 63.3 species were observed during any one year. The bird community is numerically dominated by Meadowlarks which on the average account for 37.5 percent of the birds observed. Twenty-one additional species each contributed one percent or more to total abundance and were detected all three years.

The relative abundances of several species showed clear trends over the three year period. Western Meadowlarks increased dramatically between 1976 and 1977. The Eastern Kingbird, Barn Swallow, Yellow Warbler and American Goldfinch also showed consistent increases. The dominant raptors (Red-Tailed Hawk and Sparrow Hawk) have decreased consistently since 1975. Also, a pair of Golden Eagles which nested near the census route in 1975 and 1976 were not seen in 1977. Raptors are among the species most sensitive to habitat fragmentation and their apparent numerical decline may be significant. Other species showing consistent numerical decline were the Black-Capped Chickadee, Red-Winged Blackbird, Brewer's Blackbird and Lark Bunting. At this point, the significance of these trends cannot be fully evaluated.

Overall H' decreased sharply between 1976 and 1977. This decrease was greater than typically is observed in annual fluctuations in bird communities (Järvinen and Väisänen, 1976). Evenness (J') has decreased consistently since 1975.

TABLE 7.1. AVIAN SPECIES OBSERVED ON THE ROADSIDE CENSUS IN THE COLSTRIP VICINITY DURING 1975, 1976, AND 1977*

SPECIES		Proportional Abundance $(x10^4)$		
Common Name	Scientific Name	1975	1976	1977
Canada Goose	*Branta canadensis*		2	22
Mallard	*Anas platyrhynchos*	7	12	9
Blue Winged Teal	*A. discors*			67
Turkey Vulture	*Cathartes aura*			2
Cooper's Hawk	*Accipiter cooperi*			3
Red-Tailed Hawk	*Buteo jamaicensis*	24	12	7
Rough-Legged Hawk	*B. lagopus*		1	
Golden Eagle	*Aquila chrysaetos*	5	6	
Marsh Hawk	*Circus cyaneus*	12	19	14
Prairie Falcon	*Falco mexicanus*	7	2	
Sparrow Hawk	*F. sparverius*	140	118	93
Ruffed Grouse	*Bonasa umbellus*		1	2
Sharp-Tailed Grouse	*Pedioecetes phasianellus*	24	27	68
Ring-Necked Pheasant	*Phasianus colchicus*	339	299	291
American Coot	*Fulica americana*		2	1
Killdeer	*Charadrius vociferus*	15	11	38
Common Snipe	*Capella gallinago*	2		
Upland Sandpiper	*Bartramia longicauda*		4	5

Table 7.1 Continued.

SPECIES		Proportional Abundance ($\times10^4$)		
Common Name	Scientific Name	1975	1976	1977
Solitary Sandpiper	*Tringa solitaria*			1
Northern Phalarope	*Lobipes lobatus*			5
Mourning Dove	*Zenaidura macroura*	787	775	802
Black-Billed Cuckoo	*Coccyzus erythropthalmus*		2	6
Great Horned Owl	*Bubo virginianus*		1	
Poor-Will	*Phalaenoptilus nuttallii*	2	2	12
Common Nighthawk	*Chordeiles minor*	10	9	17
Chimney Swift	*Chaetura peligica*	29	3	
Belted Kingfisher	*Megaceryle alcyon*	2	1	
Common Flicker	*Colaptes auratus*	92	143	69
Red-Headed Woodpecker	*Melanerpes erythrocephalus*	7	2	1
Hairy Woodpecker	*Dendrocopos villosus*		1	48
Eastern Kingbird	*Tyrannus tyrannus*	123	155	223
Western Kingbird	*T. verticalis*	131	87	127
Cassin's Kingbird	*T. vociferans*	10	8	
Say's Phoebe	*Sayornis saya*	22	14	17
Western Wood Pee Wee	*Contopus sordidulus*		30	9
Horned Lark	*Eremophila alpestris*	2	2	
Violet Green Swallow	*Tachycineta thalassina*		20	61
Barn Swallow	*Hirundo rustica*	107	121	153
Cliff Swallow	*Petrochelidon pyrrhonota*	228	681	320
Black Billed Magpie	*Pica pica*	17	16	37
Common Raven	*Corvus corax*	5		
Common Crow	*C. brachyrhynchos*	77	85	87
Piñon Jay	*Gymnorhinus cyanocephala*	249	162	45
Black-Capped Chickadee	*Parus atricapillus*	114	70	16
White-Breasted Nuthatch	*Sitta carolinensis*	2	1	
House Wren	*Troglodytes aedon*	5	2	45
Winter Wren	*T. troglodytes*	7	1	
Rock Wren	*Salpinctes obsoletus*		1	22
Gray Catbird	*Dumetella carolinensis*		1	6
Brown Thrasher	*Toxostoma rufum*	10	13	8
Sage Thrasher	*Oreoscoptes montanus*			3
American Robin	*Turdus migratorius*	196	176	185
Thrush		5	1	7
Mountain Bluebird	*Sialia currucoides*	39	15	14
Cedar Waxwing	*Bombycilla cedrorum*	5		
Loggerhead Shrike	*Lanius ludouicianus*	17	10	2
Starling	*Sturnus vulgaris*	19	39	43
Yellow Warbler	*Dendroica petechia*	56	131	204
Ovenbird	*Seiurus aurocapillus*	2		
Common Yellowthroat	*Geothlypis trichas*	10		1
Yellow-Breasted Chat	*Icteria virens*	17	26	37
American Redstart	*Setophaga ruticilla*	5		

Table 7.1 Continued.

SPECIES		Proportional Abundance (x10^4)		
Common Name	Scientific Name	1975	1976	1977
Western Meadowlark	*Sturnella neglecta*	3448	3457	4339
Red-Winged Blackbird	*Agelaius tricolor*	361	239	119
Northern Oriole	*Icterus galbula*	2	15	5
Brewer's Blackbird	*Euphagus cyanocephalus*	605	428	359
Common Grackle	*Quiscalus quiscula*	12	14	7
Brown-Headed Cowbird	*Molothrus ater*	140	178	103
Black-Headed Grosbeak	*Pheuctious melanocephalus*		4	
Lazuli Bunting	*Passerina amoena*	22	7	2
American Goldfinch	*Spinus tristis*	56	187	173
Red Crossbill	*Loxia curvirostra*	36		22
Rufous-Sided Towhee	*Pipilo erythrophthalmus*	36	54	91
Lark Bunting	*Calamospiza melanocorys*	1165	669	382
Savannah Sparrow	*Passerculus sandwichensis*	157	180	59
Vesper Sparrow	*Pooecetes gramineus*	479	755	324
Lark Sparrow	*Chondestes grammacus*	465	475	578
Dark-Eyed Junco	*Junco hyemalis*		1	
Chipping Sparrow	*Spizella passerina*	31	14	104
Clay-Colored Sparrow	*S. pallida*	5		72
White-Crowned Sparrow	*Zonotrichia leucophrys*	5		
	H'	3.77	3.80	3.65
	J'	.63	.62	.61
	S	61	66	63
	N (x10^0)	4130	16060	8655
	# Censuses	9	21	12
	$\bar{N}$/census	458.9	764.8	721.2

*This is not an exhaustive species list for the area. It contains only those species observed by the census methods. Also, several rare, tentatively identified species have been omitted.

Quantitative changes in species composition are often expressed by the Shannon-Weaver function (H'). This is a weighted measure influenced by both the total number of species present (S) and the relative abundance of species.

Trends in H', J' and S during the breeding seasons of 1975 through 1977 are shown in Figure 7.1. Diversity (H') rises rapidly in early spring with the arrival of migrant species. It then plateaus with maximum values in early to midsummer. A gradual decline follows through September. The seasonal patterns were very similar for the three years censused.

Between year differences in across seasonal values of H' for individual sites were greater than for the overall area values in Table 7.1. It has been suggested that increased species diversity contributes positively to commumity

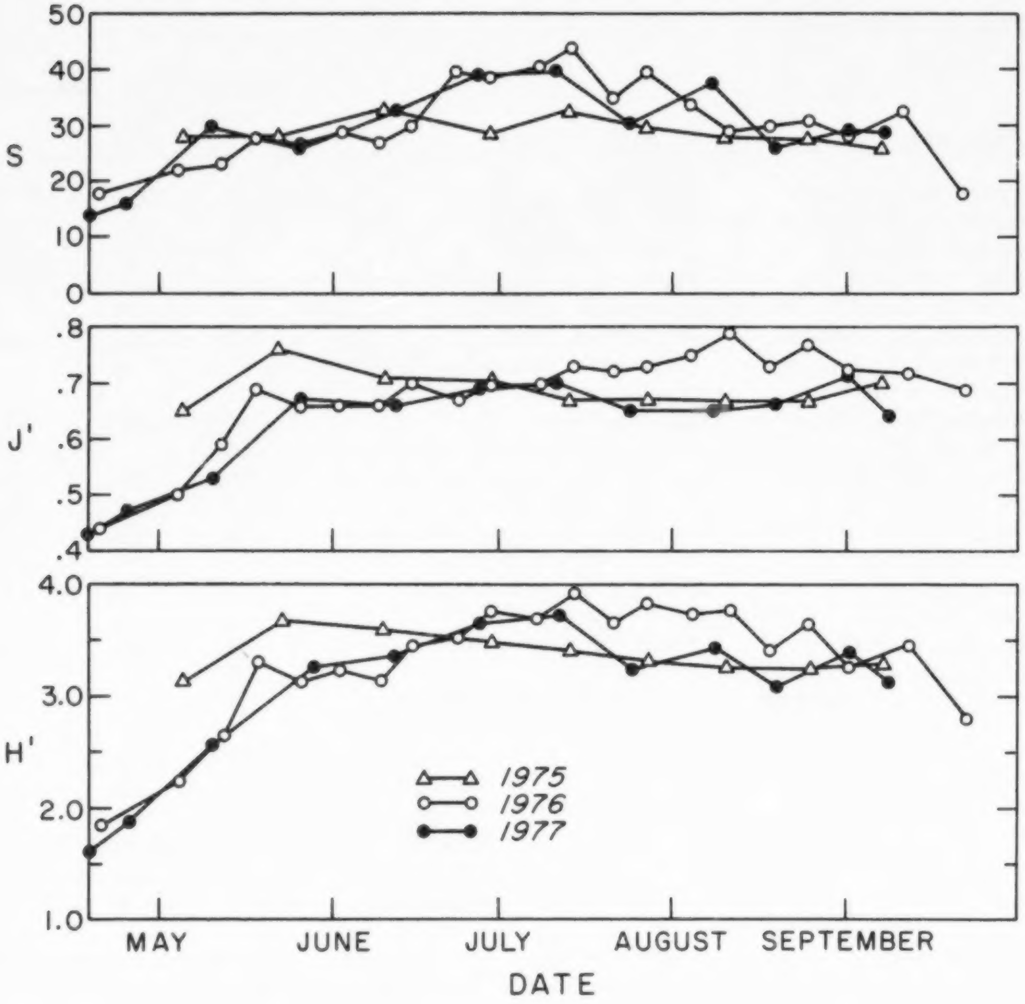


Figure 7.1. Seasonal trends in S J', and H' in the Colsrip vicinity.

stability (Woodwell and Smith, 1969). Annual fluctuations of species relative abundance patterns as reflected in H' are one indication of stability. One might expect low diversity sites to show greater between year variability in H' than high diversity sites. This is apparent, in general, with the 60 sampling sites censused. Mean across season H' for the three years censused was negatively correlated with it's standard deviation ($r = -.36$, $P < 0.01$). Low diversity sites apparently have greater annual fluctuation in species abundance patterns than high diversity sites. The mathematical nature of H' could contribute to this result. The addition of rare species to a sample with low H' usually causes a greater change than the addition of the same rare species to a sample initially yielding a high H' value.

Average across season H' and S for birds in the pine savannah southeast of Colstrip are intermediate between typical values for grasslands and those for shrublands (Table 7.2). This is expected since the sampling stations range from open grassland to forest. Average J' is lower than would be predicted by the trends for H' and S. Variability in H' and S, and J' is very similar to that found in other grassland and shrubland censuses (Tramer, 1969). Since the baseline year (1975), species richness has increased and evenness has decreased. This supports the previous observation that the bird community is becoming more highly dominated by abundant species even though new, rare species are being recorded.

It is possible to account for much of the present site variation in bird species diversity by variations in habitat. Multiple correlation analysis has been used to determine the degree of association of H' with habitat factors. For each of the sixty sampling sites, cumulative across season H' has been regressed against the proportion of grassland, proportion of pine-juniper forest, proportion of agricultural land, presence or absence of riparian habitat, presence or absence of strip mines, presence or absence of human structures, and distance from Colstrip.

TABLE 7.2. CUMULATIVE ACROSS SEASON SPECIES' DIVERSITY, EVENNESS, AND RICHNESS

	Species Diversity* (H')	Evenness* (J')	Species Richness* (S)
Colstrip 1975	2.47±0.16	0.73±0.02	10.95±0.98
Colstrip 1976	2.73±0.09	0.71±0.03	14.45±0.86
Colstrip 1977	2.56±0.18	0.67±0.03	14.21±1.21
Typical Grassland ‡	1.93±0.24	0.84±0.03	5.74±1.00
Typical Shrubland ‡	3.14±0.16	0.85±0.24	14.08±2.31

* Cumulative across season values averaged for 60 sampling sites ± 2 standard errors.

‡ from Tramer (1969)

The results of the correlation analyses are shown in Table 7.3. The multiple regression equations were statistically significant for all three years ($P < 0.005$ in all cases) indicating that the habitat variables selected were significantly associated with H'. Presence of human structures, the proportion of pine-juniper coverage, the proportion of area cultivated, the presence of riparian habitat, and distance from Colstrip were positively associated with species diversities. The presence of strip mining and the proportion of native grass coverage were negatively correlated with species diversity.

Over the three year study period, the presence of human structures had the highest positive correlation with diversity but the strength of this relationship has steadily decreased. The correlation with the proportion of pine coverage has increased. In all three years, regressions of H' against presence of human structures and pine coverage yielded higher F values than regressions against other possible combinations of variables. The habitat modification associated with human structures (deciduous trees, watering tanks, shrubs, etc.) are apparently favorable to enrichment of species diversity in this environment. Presence of pine-juniper forest permits greater habitat stratification than is present in grasslands and this may lead to higher diversity. Presence of riparian habitat is also strongly correlated with species diversity. It provides a qualitatively different habitat and may be the major water source for most species.

One might expect environmental impact to be related to distance from the power plant. Distance might well be a surrogate index of stress. However, in all years, distance of the sampling site from Colstrip accounted for very little of the variance in site diversity and the strength of the correlation has steadily decreased.

TABLE 7.3. PARTIAL CORRELATION OF H' WITH VARIABLE LISTED

Year	R^2	STRUC	PINE	STRIPM	RIP	GRASS	AG	DIST
1975	.54	.53	.02	-.35	.46	-.35	.31	.31
1976	.45	.43	.07	-.20	.37	-.37	.35	.16
1977	.43	.38	.12	-.10	.41	-.47	.32	.05

Key: R^2 = proportion of variabity in H' accounted for by regression against the 8 variables listed.
STRUC = presence of human structures (+/-)
PINE = proportion of site covered by Ponderosa Pine.
STRIPM = presence of strip mining activity in immediate vicinity (+/-)
RIP = presence of riparian habitat (+/-)
GRASS = proportion of site covered by native grasses
AG = proportion of site cultivated
DIST = Distance from Colstrip

Seasonal Changes in the Spatial Patterns of Bird Populations

Patterns in the use of space reflect adaptive behavioral choices made by species. These patterns may change seasonally in response to changes in habitat factors and/or changes in physiological state of the birds. We wished to develop statistical descriptive methods to summarize the spatial patterns observed. Such methods are potentially useful in quantifying a species response to environmental change.

A number of ecological and behavioral factors may underlie the observed dispersion pattern of a species (Weins, 1976; Rotenberry & Weins, 1976). The pattern may reflect adaptive behavioral mechanisms for resource allocation. These may range from rigorous territorial defense to foraging flocks and colonial nesting. If only a portion of the area sampled provides suitable habitat, individuals may tend to congregate in the suitable portions. Behavioral effects on dispersion are then superimposed on the tendency to aggregate in favorable habitat. It would be useful to be able separate the effects of resource distribution from behavioral effects on species dispersion patterns.

The ratio of the sample variance to the sample mean ($S^2/\bar{x}$) has been commonly used as an index of the dispersion pattern in plant and animal populations (Greig-Smith, 1964; Pielou 1969). If the individuals of a population are randomly distributed over the study area, the number of individuals per sample site is expected to follow a Poisson distribution, and the ratio of the variance to the mean is 1. If individuals tend to aggregate the ratio is greater than 1.

Figure 7.2 shows the seasonal changes in the $S^2/\bar{x}$ ratio from May to September for Western Meadowlark, the most abundant bird species in the Colstrip study area. The pattern is consistent for the three years. The exceptionally high peak during 1976 occured on a day when a flock of 73 meadowlarks was seen. A clumped distribution is evident at all times but varies seasonally in degree. Territorial behavior tends to disperse individuals during spring and early summer. Later in summer, the tendency to flock increases.

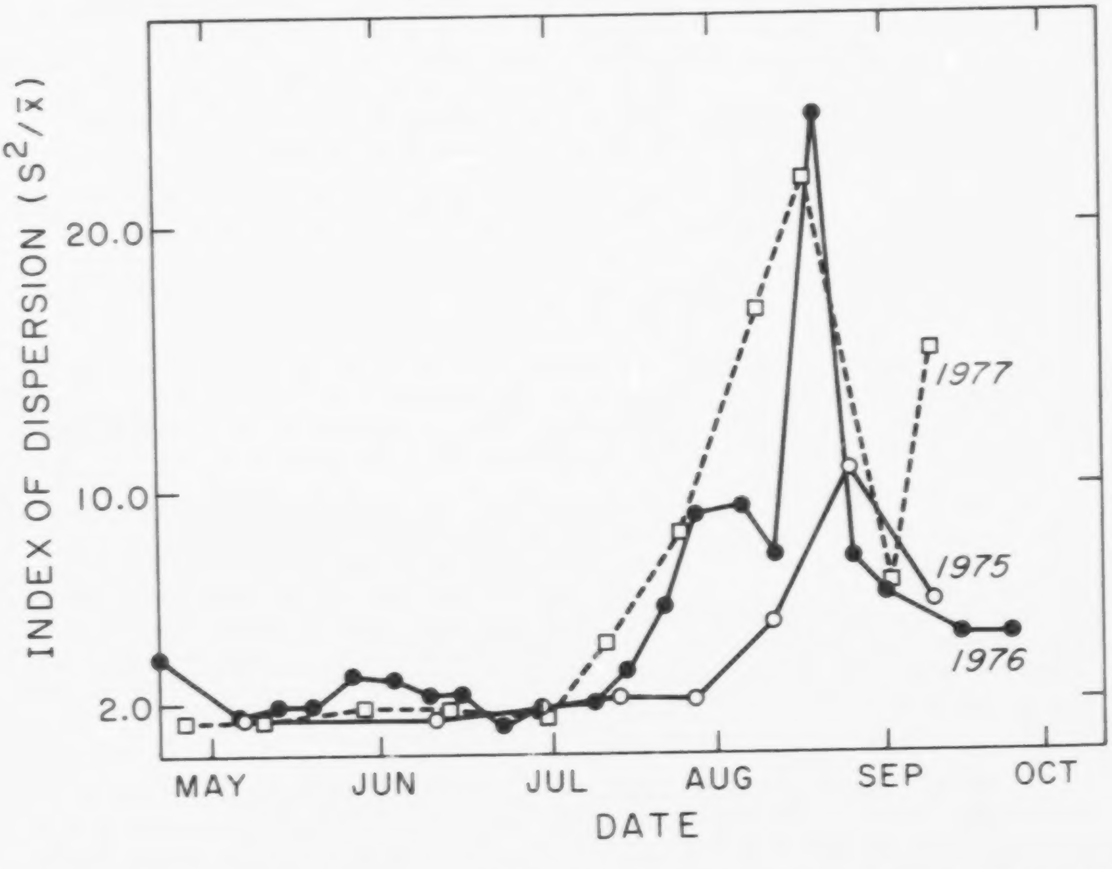


Figure 7.2. Seasonal changes in the Index of Dispersion for Western Meadowlarks.

The negative binomial distribution is often used as an empirical statistical distribution to describe animal and plant populations that are clumped (Elliot, 1971; Pielou, 1977). However, the observed distributions in the Colstrip bird censuses are often bimodal, with one mode over zero counts and the other mode representing the most common count of that species where it is observed (Figure 7.3).

The negative binomial distribution can not adequately describe such a bimodal situation. However, it can be modified to allow for added zeros. One way to interpret such a distribution, suggested by Pielou (1969), is to assume a certain proportion of the sites are not inhabited, for environmental reasons that may not be detected by the observer. Given that a site is habitable, the number of individuals observed at the site follows a negative binomial distribution. Even if a site is habitable, there may still by chance be no individuals present. The habitablility of sites can change seasonally for a bird species, as the moisture supply fluctuates, plants progress phenologically, or prey species vary in availability.

The negative binomial distribution with added zeros can be written so that the three parameters are intuitively interpretable (see Appendix 7.1 for mathematical details). Ø is the proportion of sites which are habitable, M is the expected number of individuals at a site which is habitable, and K is a parameter which indicates the degree of clumping or aggregation of the population within the habitable area. As the distribution of individuals among sites approaches a random pattern, 1/k approaches zero (*i.e.* k approaches infinity). 1/k increases (k decreases) as clumping increases.

Likelihood ratio tests showed that in almost all cases the distribution with added zeros gave a significantly better description of the data than the ordinary negative binomial model. Significance levels were calculated using the approximate chi-square distribution of twice the logarithm of the likelihood ratio (Bickel, P.J. and Doksum, K.A. 1977, p. 229).

Figure 7.3 shows observed and estimated distributions for Western Meadowlarks and Lark Buntings in spring, when the tendency to flock is low, and late summer when the largest flocks are seen. The parameters of the fitted distributions were estimated by maximum likelihood (see Appendix 7.1).

Western Meadowlark is widely distributed in the study area. Lark Bunting is patchily distributed, but is quite common at certain sites. The spring histograms clearly show the bimodal nature of the distributions. The sites with zero counts may be uninhabitable to the species, or perhaps individuals are simply congregating elsewhere. Lark buntings leave a large proportion of sites uninhabited even in late spring when no tendency to congregate or form flocks is evident. In late summer, the dramatic increase in flocking in both species is evidenced both by the heavy tails of the distributions and the increased proportion of sites with no individuals.

Strictly speaking, the parameter Ø does not imply habitability in a biological sense. A more accurate interpretation of the model is that for a proportion (Ø) of the census sites the distribution of observed birds can be approximated by a negative binomial distribution, while no birds of that

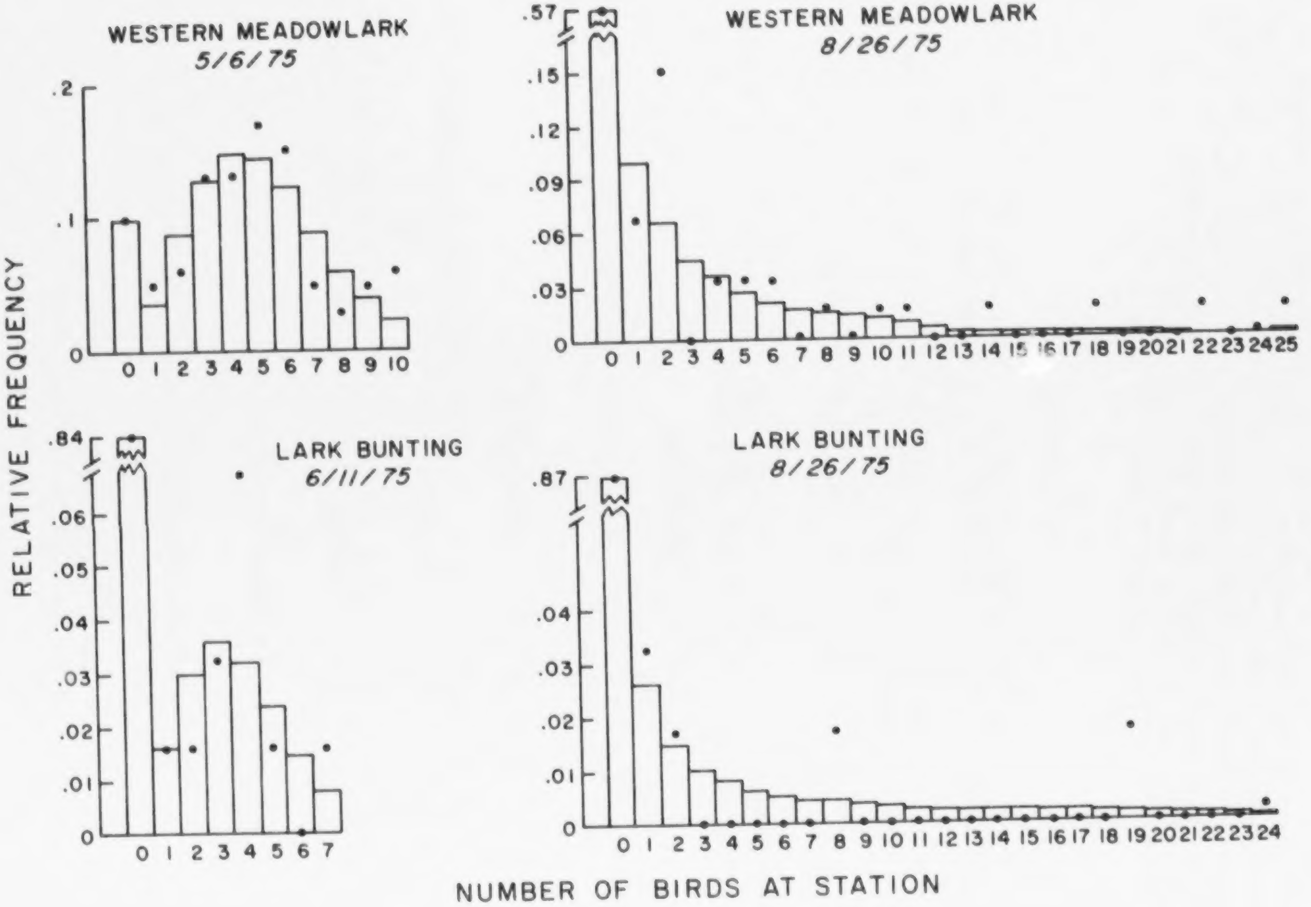


Figure 7.3. Frequency distributions of Western Meadowlarks and Lark Buntings as observed (•) and as described by the negative binomial with added zeros.

species are found on the remaining (1 - Ø) proportion of the sites. The purpose of using such a two-part statistical distribution in describing the spatial distribution of birds is to get a useful measure of changes in aggregation which takes into account the fact that some species are more restricted than others in their range within the study area. A change in spatial pattern caused by air pollution or other environmental disturbance could be detected as a change in overall density (μ), a restriction or expansion of the range of the species within the study area (Ø), or changes in social organization such as changes in territoriality or flocking behavior (k).

CONCLUSIONS

1. The bird fauna in the Colstrip vicinity is numerically dominated by Western Meadowlarks and this dominance has increased since 1975.

2. The relative abundances of raptors, blackbirds, and Lark Buntings have apparently decreased since 1975.

3. Much of the local variation in bird species diversity is correlated with habitat factors.

4. Statistical distributions with added zeros are useful in describing the spatial distributions of common bird species in the Colstrip area.

ACKNOWLEDGEMENTS

Larry Doe, Eric Preston, and John Chilgren performed the census during 1975, 1976, and 1977, respectively.

REFERENCES

Bickel, P.J. and K.A. Doksum. 1977. Mathematical Statistics; Basic Ideas and Selected Topics. Holden-Day, San Francisco.

Elliott, J.M. 1971. Some Methods for the Statistical Analysis of Samples of Benthic Invertebrates. Freshwater Biological Association, Scientific Publication no. 25.

Emlen, J. 1971. Population Densities of Birds Derived From Transect Counts. Auk, 88(2):323-342.

Greig-Smith P. 1964. Quantitative Plant Ecology. Butterworth's, Washington, D.C. 256 p.

Järvinen, O. and R. Väisänen. 1976. Between-year Component of Diversity in Communities of Breeding Land Birds. Oikos, 27(1):34-39.

Lewis, R.A., M.L. Morton, and S. Jones. 1976. The Effects of Coal-Fired Power Plant Emissions on Vertebrate Animals in Southeastern Montana. In: R.A. Lewis, N.R. Glass, and A.S. Lefohn, eds. The Bioenvironmental Impact of a Coal-fired Power Plant, 2nd Interim Report. EPA-600/3-76-013, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 140-187.

MacArthur, R.H. and J.W. MacArthur. 1961. On Bird Species Diversity. Ecology, 42(3):594-598.

Pielou, E.C. 1969. An Introduction to Mathematical Ecology. Wiley-Interscience, New York 286 p.

Pielou, E.C. 1975. Ecological Diversity. Wiley Interscience, New York. 165 p.

Pielou, E.C. 1977. Mathematical Ecology. Wiley Interscience, New York. 385 p.

Robbins, C.S. and W.T. Van Velzen. 1970. Progress Report on the North American Breeding Bird Survey. In: Bird Census Work and Environmental Monitoring, S. Svenson, ed. Bull. Ecol. Res. Comm. No. 9. Lund, Sweden. pp. 22-30.

Rotenberry, J.T. and J.A. Wiens. 1976. A Method for Estimating Species Dispersion from Transect Data. Am. Midl. Nat., 95(1):64-78.

Shannon, C.E. and W. Weaver. 1949. The Mathematical Theory of Communication. University of Illinois Press, Urbana.

Stickel, W.H. 1975. Some Effects of Pollutants in Terrestrial Ecosystems. In: Ecological Toxicology Research, A.D. McIntyre and C.F. Mills, eds. Plenum Press, New York.

Tramer, E.J. 1969. Bird Species Diversity: Components of Shannon's formula. Ecology, 50(5):927-929.

Weins, J.A. 1974. Climatic Instability and the Ecological Saturation of Bird Communities in North American Grasslands. Condor, 76(4):385-400.

Weins, J.A. 1976. Population Responses to Patchy Environments. Ann. Rev. Ecol. Syst., 7:81-120.

Woodwell, G.M. and H.H. Smith. 1969. Diversity and Stability in Ecological Systems. Brookhaven Symp. Biol. No. 22. BNL 50175. Brookhaven National Laboratory, Upton, N.Y. 264 p.

APPENDIX 7.1 The negative binomial distribution with added zeros.

The negative binomial probability density function can be written:

$$g(x) = \binom{k+x-1}{x}\left(\frac{k}{\mu+k}\right)^{k}\left(\frac{\mu}{\mu+k}\right)^{x} \qquad x=0,1,2,\ldots$$

$$\text{where} \quad \begin{matrix} k+x-1 \\ x \end{matrix} = [k\ (k+1)\ (k+2)\ \ldots\ (k+x-1)]\ \frac{1}{x!}$$

$$\text{Note: if } x=0,\ \binom{k+x-1}{x} = 1$$

The mean and variance of the distribution are

$$E(x) = \mu$$

$$\text{Var}\ (x) = \mu + \frac{\mu^2}{k}$$

As $\frac{1}{k}$ approaches zero, the variance approaches the mean and the negative binomial distribution approaches the Poisson distribution (Elliott, 1971). Thus, the estimated value of $\frac{1}{k}$ can be used as a measure of the degree to which individuals in the population are more clumped than random.

Pielou (1969) gives an interpretation of statistical distributions with added zeros in terms of habitability of sites. Suppose that census sites are either habitable or uninhabitable to a species due to factors that may be unknown to us. Individuals of the species follow the distribution in question only at the habitable sites.

The density function of the negative binomial with added zeros can be parameterized:

$$f(x) = \begin{matrix} 1-\emptyset+\emptyset\ g(x) & \text{if } x=0 \\ \emptyset\ g(x) & \text{if } x=1,2,3,\ldots \end{matrix}$$

where $\emptyset$ is the proportion of census sites that are "habitable" to that species at that time of year and $g(x)$ is the negative binomial density function.

The distribution $f(x)$ can be reparametrized as:

$$f(x) = \begin{cases} 1-\theta & \text{if } x=0 \\ \theta\, p(x) & \text{if } x=1,2,3,\ldots \end{cases}$$

where θ is the proportion of sites that have one or more birds of the species and $p(x)$ is the zero-truncated negative binomial distribution:

$$\theta = \emptyset \left(1-g(0)\right) = \emptyset \left(1- \left(\frac{k}{\mu+k}\right)^{k}\right)$$

$$p(x) = \frac{g(x)}{1-g(0)} = \binom{k+x-1}{x} \left(\frac{k}{\mu+k}\right)^{k} \left(\frac{\mu}{\mu+k}\right)^{x} \left[1- \left(\frac{k}{\mu+k}\right)^{k}\right]^{-1}$$

Maximum likelihood estimates of the parameters are obtained from census data as follows. Let $\underset{\sim}{x}$ represent the vector of observed counts for a census. For notational convenience, order the observations so all the zero counts come first.

$$\underset{\sim}{x} = (x_1, x_2, \ldots, x_m, x_{m+1}, \ldots, x_n)$$

x_i is the number of birds of that species observed at site i.

m is the number of sites where none were observed.

n is the number of sites, 60 on our survey.

The likelihood function for f(x) given this data is:

$$L\,(\theta,\ \mu,\ k;\ \underset{\sim}{x}) = \prod_{i=1}^{n} f(x_i)$$

$$= \prod_{i=1}^{m} (1-\theta) \prod_{i=m+1}^{n} \theta\, p(x)$$

$$= (1-\theta)^m\, \theta^{n-m} \prod_{i=m+1}^{n} \left\{ \left[\binom{k + x_i - 1}{x_i} \left(\frac{k}{\mu+k}\right)^k \left(\frac{\mu}{\mu+k}\right)^{x_i} \right] \left[1 - \left(\frac{k}{\mu+k}\right)^k \right]^{-1} \right\}$$

Maximization is more readily carried out with the log - likelihood function:

$$\ln\ L\,(\theta,\ \mu,\ k;\ \underset{\sim}{x}) = m \ln (1-\theta) + (n-m) \ln \theta + \sum_{i\,=\,m+1}^{n} \sum_{j=0}^{x_i-1} \ln (k+j) - \sum_{i\,=\,m+1}^{n} \ln x_i!$$

$$+ (n-m)\, k \ln k - (n-m)\, k \ln (\mu+k) + \sum_{i=m+1}^{n} x_i \ln \mu - \sum_{i=m+1}^{n} x_i \ln (\mu+k)$$

$$- (n-m) \ln \left[1 - \left(\frac{k}{\mu+k}\right)^k \right]$$

The partial derivatives used to find the values of θ, μ, and k which maximize this equation are:

$$\frac{\partial \ln L}{\partial \theta} = -\frac{m}{1-\theta} + \frac{n-m}{\theta}$$

$$\frac{\partial \ln L}{\partial \mu} = \left[\frac{1}{\mu} - \frac{1}{\mu+k}\right] \sum_{i=m+1}^{n} x_i - (n-m)\frac{k}{\mu+k} \left[1 + \frac{1}{\left(\frac{\mu+k}{k}\right)^k - 1} \right]$$

$$\frac{\partial \ln L}{\partial k} = \sum_{i=m+1}^{n} \sum_{j=0}^{x_i-1} \frac{1}{k+j} - \frac{\sum_{i=m+1}^{n} x_i}{\mu+k} + (n-m) \left[1 + \ln \left(\frac{k}{\mu+k}\right) - \frac{k}{\mu+k} + \frac{\left(\frac{\mu}{\mu+k} + \ln \left(\frac{k}{\mu+k}\right)\right)}{\left(\frac{\mu+k}{k}\right)^k - 1} \right]$$

By setting the partial derivative equal to zero, the maximum likelihood estimate for θ is seen to be $\frac{n-m}{n}$. The estimates for μ and k must be found iteratively.

The proportion of "habitable" sites (∅) can be estimated as:

$$\hat{\emptyset} = \theta / \left(1 - \left(\frac{\hat{k}}{\hat{\mu}+\hat{k}}\right)^{\hat{k}} \right)$$

Where $\hat{\emptyset}$, $\hat{\theta}$, $\hat{\mu}$, and $\hat{k}$ are maximum likelihood estimates of ∅, θ, μ, and k.

SECTION 8

BASELINE HISTOLOGY OF THE THYROID AND THYMUS GLANDS OF WESTERN MEADOWLARKS NEAR COLSTRIP

M. D. Kern and J. P. Wiggins

ABSTRACT

The objective of this part of the Colstrip Project is to provide baseline histological information concerning selected tissues of the Western Meadowlark (*Sturnella neglecta*) for use in (1) assessing the impact of pollutants on birds residing near coal-fired power plants, and (2) formulating a siting protocol for coal-fired power plants in the Great Plains region. The histology of the meadowlark's thyroid and thymus glands (collected between April and September, 1975-1977) is presented in this section. The thyroid of adult meadowlarks becomes increasingly active between April and July, as the period of reproduction concludes and just prior to the postnuptial molt, and remains highly active while molt is in progress (July through September). There are no sex-related differences in the activity of the gland. However, juveniles have more active thyroids than adults during June. Of five histological parameters examined, nuclear diameter and epithelial height appear to be the most sensitive indicators of small changes in the activity of the thyroid gland. The thymus of adult meadowlarks recrudesces during the reproductive season (May-June) and is fully developed and active when the postnuptial molt begins (July). It remains enlarged and active into September. Juvenile birds have larger and more active thymus glands than molting adults.

INTRODUCTION

A general objective of the research program at Colstrip is to identify bioindicators in the vicinity of coal-fired power plants which (1) are sensitive to perturbations in the environment, particularly to the chronic emissions of power sources, (2) exhibit obvious and/or immediate responses to pollutants, (3) can be monitored easily and inexpensively, and (4) can be used to predict the long-term impact of pollution on the ecosystem.

The program focuses on all trophic levels of the grassland ecosystem at Colstrip. The dominant consumers which are being examined in the uppermost trophic levels include the deermouse (*Peromyscus maniculatus*) and the Western Meadowlark (*Sturnella neglecta*). This section deals with the thyroid and thymus glands of the latter species.

These findings are a small segment of a larger effort to identify specific avian organs which are sensitive to low levels of toxic, but non-lethal, emissions of stationary power sources. Organs selected for study include

1. those which are *stress* indicators: the adrenal and thyroid glands

2. those involved in *immune responses*: the bursa of Fabricius, thymus, and spleen

3. those which *detoxify* pollutants: the liver, kidney, and lung

and 4. those important for the *breeding success* of meadowlarks: the reproductive organs.

We propose to develop statements about (1) how seasonal changes in organ structure reflect seasonal changes in organ function, (2) how organ structure is affected by pollution challenge, and (3) how changes in organ structure can be used to indicate existing levels of toxicity and to predict long-term population changes if exposure to pollutants persists at such levels. We anticipate that information of this nature will be useful in developing a siting protocol for coal-fired power plants in the Great Plains region. It is particularly important to gather information about terrestrial vertebrate forms such as meadowlarks because they are readily visible to the public, are likely to be immediately missed should they disappear, and because studies of *chronic* pollution stress on birds generally are few (Truhaut, 1975).

Rationale for the Project

In general, the vertebrate species which disappear earliest from polluted ecosystems are carnivores (Stickel, 1975). Since Western Meadowlarks are largely carnivorous and are the dominant avian species in the grassland ecosystem at Colstrip, Montana, they may *a priori* be expected to disappear from the vicinity of coal-fired power sources rather quickly. Unlike the members of lower trophic levels, birds accumulate pollutants, frequently for considerable periods of time, before they become visibly distressed and suddenly disappear (Stickel, 1975). It would be useful therefore by selective sampling to be able to determine (1) which avian organ-systems selectively accumulate pollutants and at what rates, as well as (2) which organ-systems are most readily damaged by pollutants, in order to anticipate when levels of these toxicants will become so high that they impair the survival of the group.

The organ-systems most likely to be affected by toxicants are those that deal directly or indirectly with them under normal circumstances. These are precisely the ones selected for study: (1) detoxifying organs, (2) immune system, (3) stress-mediating organs, and (4) reproductive organs. Birds have already proven useful as bioindicators of aerial pollution in highly industrialized areas of Japan. Doves in these areas develop lung pathologies when aerial pollution levels are 10-times lower than those necessary to produce analogous pathologies in human lungs (Lewis, Glass, and Lefohn, 1975). In effect, they provide an *early warning system* when industrial emissions approach critical toxic levels. Birds are particularly well suited to monitor aerial emission levels because (1) their lungs are structurally analogous to the high volume samplers used in aerial pollutant scavenging, and (2) they are far-ranging organisms which sample air over considerable distances from power sources. Since they also feed in the same areas, examination of their livers and kidneys provides a method of separately, but concurrently, monitoring the accumulation (and damage) of pollutants obtained through the diet, as well as those which lodge in the lungs and are obtained from the surrounding air.

Vertebrate responses to pollution challenge vary seasonally and are a function of such things as species, age, sex, social rank, and physiological status. It is therefore important to begin by describing the normal structure of avian organ-systems as a reference with which to compare the same organ-systems after birds have been exposed to the emissions of coal-fired power plants.

Major Objectives of the Avian Histological Project

In the larger view, the major objectives of the avian histological project are

1. To evaluate the normal histology of selected organs of Western Meadowlarks collected during the *preoperational* phase of the Colstrip Project. Such organs were collected between 1975 and 1977. The power plant at Colstrip has been operating since the summer of 1976 at levels ranging between 30 and 90% of peak capacity. Organs collected during 1976 and 1977 are considered preoperational in kind because they come from areas either not polluted at all or only minimally polluted.

2. To evaluate the histology of the same selected organs of Western Meadowlarks collected during the *postoperational* phase of the Colstrip Project (1978-1979), or from ecologically similar sites near power sources elsewhere in the North Central Great Plains.

3. To identify organ-systems of the Western Meadowlark which are reliable and sensitive indicators of air quality and which will be particularly useful biomonitors of emissions from coal-fired power plants.

4. To integrate histological measurements with other information available for meadowlarks taken at Colstrip in order to identify correlations which may be useful as (1) bioindicators of pollution challenge, and/or (2) predictors of the cumulative effects of pollutants on birds in general. Much ancillary information on meadowlarks is currently stored in the Information Storage and Retrieval System at the Corvallis Environmental Research Laboratory. It contains information about body and organ weight; body molt; carcass composition and caloric content; together with data on the breeding biology, ectoparasites, and lesions of meadowlarks collected during 1975-1977. The data bank also contains information about seasonal changes in weather and air quality at Colstrip. Additional information will be added to the system during the upcoming postoperational phase of the program.

In the narrower view of the vertebrate program at Colstrip, only objective 1 and part of objective 4 will be met.

Progress to-Date

Microscopic slides have been prepared from most of the meadowlark tissues collected during the preoperational phase of the Colstrip Project and are on hand for evaluation. To-date, we have surveyed all thyroid glands collected prior to July 1977, and many of those collected in July-September 1977. (We have not yet received all tissues collected during the latter months of 1977.) In addition, we are midway through the thymus glands collected preoperationally; and have begun work on liver and adrenal glands. On the following pages, we present a progress report concerning the quantitative and descriptive histology of the thyroid gland and preliminary remarks concerning the thymus.

Thyroid Gland

Studies of the avian thyroid gland are numerous (all but the most recent are reviewed by Falconer, 1971; Assenmacher, 1973; and Ringer, 1975; more recent studies include those of Höhn and Braun, 1977). These illustrate the importance of the thyroid gland in

1. Normal somatic growth

2. Calorigenesis.--Not only is the thyroid necessary for the maintenance of normal metabolism and concurrently for the production of heat in birds, but in many cases seasonal changes in its activity are inversely related to changes in ambient temperature (see, for example, Burger, 1938; Höhn, 1950; Davis and Davis, 1954; Wilson and Farner, 1960). High ambient temperatures, for example, reduce the secretory activity of the thyroid gland of domestic fowl, whereas low ambient temperatures have the opposite effect (reviewed by Falconer, 1971; and Ringer, 1975).

3. Molt.--Several studies illustrate the fact that thyroid activity increases shortly before or during periods of seasonal molt (reviewed by Payne, 1972; and Assenmacher, 1973). This is particularly well shown by mallards. The thyroid activity increases one month earlier in drakes

than in hens: the male also begins to molt one month earlier than the female (Höhn, 1961). On the other hand, the molt of several species occurs without a change in thyroid activity (reviewed by Payne, 1972). Furthermore, thyroidectomy may or may not abolish seasonal molts; and injections of thyroxine may or may not precipitate a molt. As Payne (1972, p. 128) says " . . . thyroid activity seems to be related to molt, but the apparent role of the thyroid in molt differs from species to species". One manner in which the gland *is* involved in feather replacement is that it stimulates cell division and growth within feather papillae (Voitkevich,1966; Höcker, 1967).

4. Reproduction.--As with molt, the role of the thyroid in reproductive activities varies considerably from one avian species to another. In some (domestic fowl, House Sparrows, Baya Weavers), annual gonadal recrudescence apparently requires thyroxine. In others (European Starlings and several subtropical Indian finches), the thyroid is not necessary for gonadal growth. In fact, thyroid activity diminishes during the breeding season of some species (Red Fodies, White-crowned Sparrows, Spotted Munias). In still other cases, however, it increases near the end of the reproductive period (European Starlings, European Blackbirds, Mallards, Wood Pigeons) and is apparently responsible for gonadal regression (Baya Weavers and Mannikins). This complex subject has recently been reviewed by Assenmacher (1973).

5. Migration.--The role of the thyroid in the control of migratory behavior and premigratory fattening is unclear (Berthold, 1975). Although thyroid activity is elevated in some species during migration (Putzig, 1938; George and Naik, 1964) and injections of thyroxine induce migratory-like behavior in some forms (Merkel, 1938), the gland probably does not play a principal regulatory role in this seasonal event.

If the thyroid gland is a major regulator of any one of the above activities in meadowlarks, and if it is sensitive to pollutants, then upsets in its activity will be translated into aberrancies in seasonal cycles. Environmental perturbations can alter thyroid activity within 7-14 days (Gelineo, 1955; Hahn, Ishibashi, and Turner, 1966) and *histological* features of the gland sometimes change before mensurable changes in glandular activity occur (Saatman and van Tienhoven, 1964). It may accordingly be possible to predict the timing and magnitude of altered seasonal events secondary to pollutant-induced changes in thyroid function simply by evaluating thyroid histology on a routine basis.

With this in mind, there are several normal correlations which need to be described between thyroid activity and other seasonal events of meadowlarks. These will be exhaustively addressed once the data for July-September, 1977, become available to us. They include correlations between various histological indices of thyroid activity (described below) and seasonal cycles of body weight, body fat, molt, reproduction, adrenocortical activity, and ambient temperature.

Thymus Gland

It is now well established that the avian thymus is a central lymphoid organ and the site of development of T-dependent lymphocytes which (1) migrate to peripheral organs, such as the spleen, and (2) are responsible for cell-mediated immune responses. T-dependent lymphocytes produce cytotoxic substances called *lymphokines* which destroy foreign bodies and are responsible for the rejection of skin grafts and for hypersensitivity reactions (the topic of avian immunity is reviewed by Cooper *et al.*, 1966; and more recently by Moticka, 1975; a thorough general discussion of this subject is also presented by Raviola, 1975*a*). Lymphokines also stimulate macrophages (which ingest and destroy foreign materials) and B-dependent lymphocytes (which produce antibodies).

Until recently it was thought that the avian thymus, like its mammalian counterpart, involuted when a bird reached sexual maturity (Hodges, 1974). However, it is now apparent that this organ recrudesces periodically in many species coincident with periods of molt and/or reproduction. This occurs in Ring-necked Pheasants (Anderson, 1970); mallards, House Sparrows, and American Robins (Höhn, 1956); Yellow-vented Bulbuls (Ward and D'Cruz, 1968); Red-billed Diochs (Bacchus and Kendall, 1975; Kendall, 1975; Ward and Kendall, 1975); and even occasionally in domestic fowl (Payne, 1971).

The thymus is also a site of formation of red and white blood cells and it has been suggested that by recrudescing it provides (1) lymphoid cells to deal with the stress associated with molt (Anderson, 1970), or (2)erythrocytes required for the rapid somatic growth of immature birds; the rapid recovery of body lipids and proteins which are depleted during breeding; and to nourish the growing feathers during periods of molt (Bacchus and Kendall, 1975; Ward and Kendall, 1975).

The thymus of immature birds is particularly sensitive to stress and will involute prematurely under conditions which produce hypertrophy of the adrenal cortex and production of adrenocortical hormones (Payne, 1971). This organ is therefore potentially useful for determining if pollutants from coal-fired power plants selectively affect young birds. Although not reported in the literature, it is plausible that pollutants of this kind may even prevent or abbreviate the recrudescence of the adult thymus gland. In either case, changes which are deleterious to the whole bird will probably appear initially as structural changes of thymic tissue.

At Colstrip, the wet weight of the meadowlark's thymus increases annually just before the postnuptial molt takes place (Preston and Lewis, 1978). Whether these changes in weight reflect changes in glandular activity needs to be determined. We are now analyzing the thymuses of the meadowlarks collected on site between 1975 and 1977 with this end in mind.

MATERIALS AND METHODS

Histological Measures of Thyroid Function

We have examined five histometric indicators of thyroid activity: (1) follicle size, (2) the number of follicles per unit area of thyroid gland, (3) the colloid content of the gland, (4) the diameter of nuclei in epithelial cells lining the follicles, and (5) the height of these epithelial cells.

We have assumed that the microscopic sections of the thyroid gland come from its center and represent conditions typical of the whole gland. We have also assumed that an *active* thyroid gland is characterized by small follicles containing little colloid (and therefore relatively large numbers of follicles per unit area), large epithelial nuclei, and a high follicular epithelium; whereas *inactive* glands feature large follicles distended with considerable colloid (and therefore relatively small numbers of follicles per unit area), flattened epithelial nuclei, and a squamous follicular epithelium. This functional interpretation of the gland's histology is consistent with the views of virtually all previous investigators.

One or more of our methods have been employed successfully in earlier studies of the avian thyroid gland (Burger, 1938; Voitkevich, 1966; von Faber, 1967; Erpino, 1968; Ljunggren, 1968; Raitt, 1968; Payne and Landolt, 1970; Höhn and Braun, 1977). Other methods are sometimes used, including calculation of the per cent of the total gland consisting of epithelium and stroma (Wilson and Farner, 1960; Höhn and Braun, 1977) or activity scales based on nuclear diameter and epithelial height (Ljunggren, 1968). All appear to adequately measure the activity of thyroid tissue and we arbitrarily settled on the five listed above. For each indicator, we used Stein's formula to determine how many observations should be made to demonstrate significant differences at the 5% level (Steel and Torrie, 1960, p. 86).

Follicular size was determined by measuring the maximal diameter of 10 randomly selected follicles in sections of each gland, using an ocular micrometer and magnifications of 400.

To determine the number of follicles per unit area of gland (*follicular density*), we counted the number of complete follicles positioned within an ocular grid in 20 randomly selected fields within the section. The magnification used was 400 and the area of tissue encompassed by the grid was 0.056 mm^2.

The *colloid content* of each gland is a single visual estimate of the volume of secretion in the entire gland, on a scale of 0 to 5, in which colloid may be absent (rating of 0), or present in trace (1), small (2), moderate (3), large (4), or very large (5) amounts. Estimates were made after scanning the section at a magnification of 40.

In order to determine the average *nuclear diameter* of epithelial cells in each gland, 20 nuclei were randomly selected from the section and measured with an ocular micrometer at a magnification of 1000. Nuclei in columnar and cuboidal epithelia are commonly spherical and accordingly nuclear diameter is constant wherever measured; however, in squamous epithelia the nuclei are either oval or distinctly flattened and have maximal and minimal diameters. We therefore used a measure of nuclear size which includes the maximum and minimum diameters of the nucleus. In this case,

$$\text{Nuclear Diameter} = \frac{(\text{maximum diameter}) + (\text{minimum diameter})}{2}$$

The heights of 20 epithelial cells randomly selected from follicles in the center of each section were averaged to obtain an index of *epithelial height* for each gland. Measurements were made at magnifications of 1000.

Wherever appropriate, we evaluated the results statistically by analysis of variance, the Student's *t*-test, linear regression, or Duncan's multiple range test with Kramer's modification for samples with unequal numbers of observations (Duncan, 1955; Kramer, 1956; Steel and Torrie, 1960).

Histological Measures of Thymus Function

We are using seven histological indicators to evaluate thymic activity. These are derived from parameters used in earlier studies of the avian thymus (Höhn, 1956; Ward and D'Cruz, 1968; Anderson, 1970; Bacchus and Kendall, 1975; Kendall, 1975). They include (1) the mitotic activity of the gland, (2) the presence or absence of a distinct cortex, (3) the cross-sectional area of cortex and/or medulla, (4) the number and morphology of Hassal's corpuscles, (5) the size of large cysts associated with Hassal's corpuscles, (6) the types and relative numbers of cells in the thymic cortex, and (7) the number of extravascular erythrocytes in the cortex.

The *mitotic activity* of each thymus is estimated after scanning the cortex (if present) or entire section (in the absence of a cortex) at magnifications of 400 for mitotic figures. Activity is rated on a scale of 0 to 5, in which mitotic figures may be absent (rating of 0), or present in small (1), mild (2), moderate (3), large (4), or very large (5) numbers. Kendall (1975) uses this index to identify enlarging thymus glands selectively: in queleas, the mitotic activity is quite high in enlarging glands, but low in fully developed, regressing, or fully regressed glands.

Differentiation of thymic tissue is quantified in each lobe of the gland by assigning the number *1* to the lobe if it has a *distinct cortex*, and the number *2* if the cortex is absent. The overall condition of each gland is the average of individual numerical values assigned to each of its lobes. The presence of a cortex is the indicator most frequently used to determine whether the thymus is functional or not. A cortex is present in enlarging, fully enlarged, and regressing glands, but not in fully regressed glands (Höhn, 1956; Ward and D'Cruz, 1968; Anderson, 1970; Bacchus and Kendall, 1975).

No measurements of the *area of the cortex* have been reported in the literature to-date. We suggest that variations in the cross-sectional area of this region may reflect its lymphopoietic activity. Areas of the cortex, medulla, and the total cross-sectional area of each section are obtained by tracing the image of each lobe and its zones onto a piece of paper, using a camera lucida and magnifying the section by a factor of 4.45. A planimeter is then used to measure the areas of cortex, medulla, and whole lobe on the tracing. The latter can be converted into areas of tissue without difficulty because 1.0 cm^2 of the tracing ≈ 0.0505 cm^2 of tissue. Since these areas are measured in a *single* section of each lobe of the thymus, we necessarily assume that the section comes from the center of the lobe and that conditions here are representative of conditions in the entire lobe. The error is most likely minimal anyway because the proportions of cortex and medulla for each bird are average values based on measurements in several lobes of the gland.

All *Hassal's corpuscles* in the section are counted and classified as "early" or "late" on the basis of their morphology. These designations come from Bacchus and Kendall (1975) and are fully described in the following section of this paper.

The function of Hassal's corpuscles is not known. They may actively remove cells from the thymus under specific conditions (Blau, 1967), or they may be nothing more than degenerating masses of thymic cells (Raviola, 1975*b*). However, they are a constant feature of the gland and their numbers increase when it involutes. In queleas, regressing glands contain large numbers of late corpuscles, whereas enlarging glands contain few-to-none (adult birds) or only early corpuscles (immature birds) (Bacchus and Kendall, 1975). Censuses of these corpuscles may permit us to distinguish enlarging from regressing thymuses.

We also measure the *maximal diameter of the largest cyst* associated with each late Hassal's corpuscle, using an ocular micrometer and magnifications of 400; and make an estimate of the most common *number of cysts* in the late corpuscles within each lobe, again at magnifications of 400. We suggest that these enumerations may provide a means of distinguishing regressing from fully regressed thymuses.

In addition, we make *censuses of the cells in the cortex* of the meadowlark's thymus. The census data are still preliminary so we have not included them in this paper. Nevertheless, we present the census method and the rationale for its use below.

The method is a modification of that used by Kendall (1975). We identify and count the number of cortical cells which occur in five randomly selected quadrants of an ocular grid. One corner of the grid is placed on the capsule of the thymus. All of the cells in the central plane of focus in the five quadrants are then identified and counted. Only cells completely enclosed by the edges of the quadrant are included. Censuses are made under oil at magnifications of 1500. To avoid bias, randomly selected areas of cortex on both sides of each lobe are used in the census. We also avoid areas of cortex containing large blood vessels in order to minimize the number of intravascular cells in the data; however, small capillaries frequently occur in the quadrants and an occasional blood cell from one of them is incorporated into the results. A total of six areas of cortex (30 quadrants) are evaluated per lobe of each gland. This amounts to 153 μm^2 of cortex (each quadrant encloses $\sim$ 5.1 μm^2 of tissue).

Kendall (1975) uses the cell populations in the cortex of queleas to distinguish between enlarging, fully developed, and regressing thymuses. Mitotic figures, for example, are only numerous in the enlarging adult gland. Lymphocytes are more numerous in enlarging glands than in regressing or regressed ones. On the other hand, enlarging glands contain fewer cortical erythrocytes than enlarged, regressing, or regressed ones. Finally, the total number of cells in the cortex is conspicuously reduced in regressing glands. Although Kendall's conclusions are based on a relatively small number of adult queleas, they suggest that cortical cell populations can be used to determine the functional status of the thymus and we have therefore applied this census technique to meadowlark tissues.

Since it has been suggested that the thymus has an erythropoietic function (Bacchus and Kendall, 1975; Ward and Kendall, 1975), we also make a visual estimate of the *number of erythrocytes* free in the cortical tissue of each lobe, after scanning the section at a magnification of 400. Erythrocytes may be absent (in which case the lobe is assigned a rating of 0), or present in small (1), mild (2), moderate (3), large (4), or very large (5) numbers. We have just begun to make these estimates and accordingly do not include them in the following section of this paper.

For each index of thymic activity, we used Stein's formula to determine the number of observations necessary to obtain statistical differences at the 5% level (Steel and Torrie, 1960, p. 86).

RESULTS AND DISCUSSION

Normal Histology of the Thyroid Gland of Western Meadowlarks

The histological organization of the meadowlark's thyroid gland is typical of that found in birds generally (see descriptions in such references as Falconer, 1971; and Assenmacher, 1973). The gland consists of numerous secretory follicles laden with more or less colloid and lined with a simple layer of epithelial cells, the height of which varies according to the activity of the gland (see above). Variable amounts of stromal material occur between the follicles and consist of connective and vascular tissue.

Variations in the Histology of the Thyroid Gland of Western Meadowlarks Between April and September

Data concerning monthly changes in thyroid histology, together with information concerning the effects of year, sex, and age on the gland, appear in Tables 8.1-8.3. There were few differences between data collected

TABLE 8.1. MONTHLY CHANGES IN THE HISTOLOGY OF THE THYROID GLAND OF ADULT WESTERN MEADOWLARKS (*Sturnella neglecta*) AT COLSTRIP, MONTANA

Year	Month	n	Nuclear Diameter[1,2] (μm)	Epithelial Height[1,2] (μm)	Follicular Diameter[1,2] (μm)	Follicular Density[1,2] (follicles/mm²)	Colloid Content of Follicles[1,2,3]
1976	Apr	22	3.19 ± 0.10 ab	3.64 ± 0.30 a	36.4 ± 1.9 a	644.6 ± 32.1 a	1.76 ± 0.06 ab
	May	16	3.24 ± 0.12 ab	4.21 ± 0.35 ab	37.7 ± 2.2 ab	657.1 ± 37.5 a	1.74 ± 0.07 ab
	Jun	5	3.76 ± 0.21 b	5.28 ± 0.63 abc	39.1 ± 3.9 ab	508.9 ± 67.9 abc	1.63 ± 0.12 ab
	Jul	3	3.67 abc	4.93 abc	43.7 ab	428.6 abc	1.58 ab
	Aug	5	3.82 ± 0.21 bc	5.36 ± 0.63 abc	33.5 ± 3.9 ab	503.6 ± 67.9 abc	1.43 ± 0.12 a
	Sep	2	3.30 ab d	3.65 abc	39.5 ab	537.5 abc	1.87 ab
1977	Apr	25	3.07 ± 0.09 a	3.78 ± 0.28 a	45.0 ± 1.8 b	573.2 ± 30.4 abc	1.87 ± 0.06 b
	May	62	3.29 ± 0.06 ab	4.74 ± 0.18 b	37.3 ± 1.1 a	610.7 ± 19.6 ab	1.61 ± 0.04 a
	Jun	55	3.49 ± 0.06 b	5.24 ± 0.19 b	36.7 ± 1.2 a	562.5 ± 19.6 abc	1.64 ± 0.04 a c
	Jul	16	3.98 ± 0.12 cd	5.94 ± 0.35 c	40.7 ± 2.2 ab	535.7 ± 37.5 abc	1.83 ± 0.07 ab
	Aug	7	4.49 ± 0.18 c	5.84 ± 0.53 b	48.5 ± 3.3 b	423.2 ± 57.1 c	2.03 ± 0.10 b
	Sep	9	4.39 ± 0.16 c	5.89 ± 0.47 b	45.7 ± 2.9 ab	433.9 ± 50.0 ab	1.92 ± 0.09 bc

[1] Values in these columns are means ± SEM; data for Jul-Sep 1977 are incomplete

[2] Means in each column are statistically different ($P < 0.01$) from each other when *not* followed by the same letter (Duncan's multiple range test with Kramer's modification for groups with unequal numbers of observations)

[3] The amount of colloid was rated on a scale of 0 to 5 (none to abundant) for each gland. The data appear in this column as $\sqrt{p}$ where p is a number between 0 and 5. The square root transformation is necessary for parametric analysis of ordinal data (Steel, R.G.D., and J.H. Torrie. 1960. Principles and Procedures of Statistics with Special Reference to the Biological Sciences. McGraw-Hill Book Co., Inc., New York. p. 157).

TABLE 8.2. MONTHLY CHANGES IN THE HISTOLOGY OF THE THYROID GLAND OF ADULT WESTERN MEADOWLARKS (*Sturnella neglecta*) AT COLSTRIP, MONTANA - 1976 AND 1977 DATA COMBINED WHERE POSSIBLE

Sampling Period	n	Nuclear Diameter^{1,2} (µm)	Epithelial Height^{1,2} (µm)	Follicular Diameter^{1,2} (µm)	Follicular Density^{1,2} (follicles/mm^2)	Colloid Content of Follicles^{1,2,3}
1976 Apr	22	3.12 ± 0.07 a	3.17 ± 0.21 a	36.4 ± 1.9 a	607.1 ± 21.4 a	1.82 ± 0.04 bc
1977 Apr	25			45.0 ± 1.8 b		
1976 1977 May	78	3.28 ± 0.05 a	4.63 ± 0.16 b	37.4 ± 1.0 a	619.6 ± 17.9 a	1.63 ± 0.04 a
1976 1977 Jun	60	3.51 ± 0.06 b	5.24 ± 0.18 bc	36.9 ± 1.3 a	558.9 ± 19.6 ab	1.64 ± 0.04 ab
1976 1977 Jul	19	3.93 ± 0.11 cd	5.78 ± 0.32 c	41.2 ± 2.0 ab	519.6 ± 33.9 ab	1.79 0.06 abc
1976 Aug	5	4.21 ± 0.13 cd	5.64 ± 0.41 bc	42.2 ± 2.5 ab	457.1 ± 25.0 b	1.43 ± 0.12 a
1977 Aug	7					2.03 ± 0.10 c
1976 Sep	2	3.30 abc	5.48 ± 0.43 bc	44.6 ± 2.6 ab	453.6 ± 44.6 b	1.91 ± 0.08 c
1977 Sep	9	4.39 ± 0.16 d				

[1] Values in the columns are means ± SEM; data for Jul-Aug 1977 are incomplete.

[2] Means in each column are statistically different ($P < 0.01$) from each other when *not* followed by the same letter (Duncan's multiple range test with Kramer's modification for groups with unequal numbers of observations).

[3] The amount of colloid was rated on a scale of 0 to 5 (none to abundant) for each gland. The data appear in this column as $\sqrt{p}$ where p is a number between 0 and 5. The square root transformation is necessary for parametric analysis of ordinal data (Steel, R.G.D., and J.H. Torrie. 1960. Principles and Procedures of Statistics with Special Reference to the Biological Sciences. McGraw-Hill Book Co., Inc., New York. p. 157).

TABLE 8.3. AGE- AND SEX-RELATED DIFFERENCES IN THE HISTOLOGY OF THE THYROID GLAND OF THE WESTERN MEADOWLARK (*Sturnella neglecta*)

Sampling Period	Age-Sex[1,2]	n	Nuclear Diameter[3,4] (m)	Epithelial Height[3,4] (m)	Follicular Diameter[3,4] (m)	Follicular Density[3] (follicles/mm^2)	Colloid Content of Follicles[3,5]
Apr 1976–1977	Ad - M	41	3.14 ± 0.07	3.72 ± 0.22	41.4 ± 1.4	600.0 ± 23.2	1.83 ± 0.04
	Ad - F	6	2.98 ± 0.19	3.63 ± 0.57	38.1 ± 3.6	648.2 ± 62.5	1.76 ± 0.11
May 1976–1977	Ad - M	44	3.27 ± 0.07	4.68 ± 0.21	38.0 ± 1.3	603.6 ± 23.2	1.64 ± 0.04
	Ad - F	34	3.30 ± 0.08	4.57 ± 0.24	36.6 ± 1.5	641.1 ± 26.8	1.63 ± 0.05
Jun 1976–1977	Ad - M	40	3.44 ± 0.10 a	5.19 ± 0.25 a	36.8 ± 1.3 a	550.0 ± 21.4	1.66 ± 0.05
	Ad - F	20	3.65 ± 0.14 a	5.35 ± 0.35 a	37.2 ± 1.8 a	578.6 ± 30.4	1.60 ± 0.06
	Ju - M	3	4.43 ab	8.17 b	24.9 b	523.2	1.38
	Ju - F	5	4.62 ± 0.28 b	8.20 ± 0.70 b	30.0 ± 3.7 b	457.1 ± 58.9	1.46 ± 0.13
Jul 1976–1977	Ad - M	6	3.43 ± 0.25	5.50 ± 0.57	43.0 ± 3.6	391.1 ± 53.6	1.74 ± 0.12
	Ad - F	13	4.15 ± 0.17	5.91 ± 0.43	40.4 ± 2.3	532.1 ± 37.5	1.81 ± 0.08
	Ju - M	11	4.11 ± 0.19	6.33 ± 0.47	36.2 ± 2.5	467.9 ± 39.3	1.71 ± 0.09
	Ju - F	1	4.80	8.00	29.2	594.6	1.42
Aug 1976–1977	Ad - M	4	3.93 ± 0.31	5.25 ± 0.78	39.9 ± 4.1	428.6 ± 66.1	1.68 ± 0.14
	Ad - F	8	4.35 ± 0.22	5.84 ± 0.55	43.4 ± 2.9	471.4 ± 46.4	1.83 ± 0.10
	Ju - M	10	4.22 ± 0.20	5.87 ± 0.50	41.2 ± 2.6	455.4 ± 41.1	1.97 ± 0.09
	Ju - F	12	4.59 ± 0.18	6.76 ± 0.45	39.2 ± 2.4	451.8 ± 37.5	1.64 ± 0.08
Sep 1976–1977	Ad - M	4	4.10 ± 0.31	5.20 ± 0.79	39.6 ± 4.1	546.4 ± 66.1	1.78 ± 0.14
	Ad - F	7	4.24 ± 0.24	5.64 ± 0.60	47.4 ± 3.1	400.0 ± 50.0	1.99 ± 0.11
	Ju - M	19	4.02 ± 0.14	5.17 ± 0.36	40.7 ± 1.9	525.0 ± 30.4	1.87 ± 0.07
	Ju - F	17	4.10 ± 0.15	5.52 ± 0.38	40.1 ± 2.0	489.3 ± 32.1	1.84 ± 0.07

[1] Data for Jul-Sep 1977 are not yet complete.

[2] Ad = adult; Ju = juvenile; M = male; F = female.

[3] Values in these columns are means ± SEM. No statistically significant differences exist between age-sex categories within any month except Jun.

[4] Means in Jun differ at the 1% level of significance if *not* followed by the same letter (Duncan's multiple range test with Kramer's modification for groups with unequal numbers of observations).

[5] The amount of colloid was rated on a scale of 0 to 5 (none to abundant) for each gland. The data appear in this column as $\sqrt{p}$ where p is a number between 0 and 5. The square root transformation is necessary for parametric analysis of ordinal data (Steel, R.G.D., and J.H. Torrie. 1960. Principles and Procedures of Statistics with Special Reference to the Biological Sciences. McGraw-Hill Book Co., New York. p. 157).

in any month during 1976 and data from the same month in 1977 (Table 8.1) and it was therefore possible in most cases to lump data for the two years (Table 8.2).

The following trends are evident in the data:

1. Nuclear diameter, epithelial height, and colloid content of thyroid follicles are the most sensitive histometric indicators of *small* changes in thyroid activity (Tables 8.1 and 8.2).

2. The significant changes which take place in nuclear and follicular diameter, epithelial height, and colloid content of *adult* thyroid glands between April and September (Table 8.2) suggest that thyroid activity increases during the April-June period and is high in the July-September period (at the time of the postnuptial molt).

3. No sexual differences exist in the activity of the adult or juvenile thyroid gland (Table 8.3).

   (In some avian species, notably mallards (Höhn, 1949) and Wood Pigeons (Ljunggren, 1968), the male gland is consistently heavier than that of the female. In Wood Pigeons, females also have more follicles, but less colloid than males during the breeding season. On the other hand, sexual differences are absent in Gambel Quail (Raitt, 1968), as they appear to be in Western Meadowlarks.)

4. Age-specific differences in thyroid activity exist only in June, when young birds are fledging. At this time, differences in nuclear diameter, epithelial height, and follicular diameter indicate that the juvenile thyroid is more active than the adult gland (Table 8.3). This is not surprising given that (1) the thyroid is involved in feather growth and thermoregulation, and (2) the feathers of recently fledged meadowlarks are still growing so that their insulation value is limited.

5. Significant correlations exist between every pair of histometric indices we used to monitor the activity of *adult* thyroid glands. However, many of the correlations are weak ($r < \pm 0.45$). The most highly correlated pairs of measures are

   a. nuclear diameter with epithelial height: $r = +0.81$ ($P < 0.01$; df = 180)

   b. epithelial height with the colloid content of the gland: $r = -0.61$ ($P < 0.01$; df = 180)

   c. follicular diameter with the colloid content of the gland: $r = +0.61$ ($P < 0.01$; df = 180).

Normal Histology of the Thymus Gland of Western Meadowlarks

The thymus gland of the Western Meadowlark consists of a series of independent lobes closely associated with the jugular veins and the trachea.

Each lobe is a separate histological unit. We do not find the marked histological variation between lobes of a single gland which Bacchus and Kendall (1975) report in queleas. To the contrary, the lobes of any meadowlark's thymus are relatively uniform histologically.

In general, the histology of any lobe is similar to that described by Payne (1971) and Hodges (1974) for the domestic fowl, provided the additional features described by Bacchus and Kendall (1975) and Kendall (1975) for queleas are included.

The thymic parenchyma of each lobe is housed in a thin capsule of collagen. Septa extend inward from the capsule and partially subdivide the lobe into lobules. Short secondary septa occasionally arise from primary septa and further subdivide the lobules into segments. Capsule and septa are well vascularized.

The parenchyma is supported by a network of reticular cells and presumably their fibers (which are not visible in our preparations). A peripheral cortex is present in the lobule, presumably when the gland is active. It tends to be sharply demarcated from the centrally located medulla. Cells are especially numerous in the cortex. Most common here are small lymphocytes which account for 70-90% of all cortical cells in the meadowlark (preliminary census data). (Percentages of small lymphocytes in the cortex of meadowlarks are roughly the same as those found by Kendall (1975) in queleas.) Small numbers of many other cell types also occupy the cortex: medium- and large-sized lymphocytes, erythroblasts, erythrocytes, plasma cells, fibroblasts, pycnotic elements, and mitotic figures. The blood supply of this area is a network of small capillaries.

Large spaces are also occasionally present in the peripheral cortex near the capsule. They may be lymphatics or ducts of the air sac system. They are commonly empty, although some occasionally contain laminated concretions and homogenous droplets. Bacchus and Kendall (1975) found similar spaces in the thymuses of queleas and called them "clear channels".

The density of the medulla is less than that of the cortex, primarily because there are fewer cells here. However, the types of cells in the two areas are the same. On the other hand, the medulla is more highly vascularized than the cortex and contains small veins and arterioles, in addition to capillaries. Vacuolation is both common and widespread in reticular cells throughout the medulla. Most of the vacuoles are intracellular, but some large ones are not. Many contain an homogenous eosinophilic fluid. In addition, large cysts pepper this region. Some of them are ciliated, but none have ducts like those described by Bacchus and Kendall (1975). They tend to be empty.

Units called Hassal's corpuscles are a constant feature of the medulla. They are composed of large epithelioid cells with eosinophilic cytoplasm, cytoplasmic vacuoles of various sizes, and large primitive mesenchymal-like nuclei. Three varieties can be distinguished in the meadowlark's thymus:

1. *Early Hassal's corpuscles.*--These units are small and contain few or no cysts; if cysts are present, they are always empty.

2. *Late Hassal's corpuscles.*—These are larger and contain numerous cysts, of which one is generally very large and surrounded by several others of small size. Many of the cysts contain debris or cells (leucocytes and/or erythrocytes).

3. *Laminated Hassal's corpuscles.*—These units are structurally similar to the classical mammalian Hassal's corpuscle (described in Bloom and Fawcett, 1975). They consist of concentric layers of epithelioid cells encircling a large central cyst which is frequently filled with cells and/or debris.

The laminated form of corpuscle is not common in meadowlarks: each gland has only one or two such corpuscles. In contrast, numerous early and late units occur in most thymus glands. For purposes of analysis, we have lumped laminated corpuscles with the late variety.

The medulla also possesses large numbers of myoid or skeletal muscle cells. They are large, tubular elements with markedly eosinophilic, fibrillar cytoplasm and one or more peripheral nuclei. They are scattered individually throughout the medulla.

Variations in the Histology of the Thymus Gland of Western Meadowlarks Between April and September

Preliminary data concerning monthly changes in the histology of the thymus gland, together with information concerning the effects of year, sex, and age on the gland, appear in Tables 8.4-8.7. We have not finished the thymus material and accordingly no statistical analyses have been done yet.

Nonetheless, some trends appear to be emerging from the data at hand and we enumerate them here:

1. The thymus of *adult* meadowlarks recrudesces during the reproductive season (May and June), and is fully developed and active when the postnuptial molt begins (July). These tentative conclusions are supported by the following observations:

    a. the cortex began to differentiate in adult meadowlarks during May and June (Tables 8.4 and 8.6)

    b. the mitotic activity of the thymus increased perceptibly during May and June, 1976 and 1977 (Table 8.6)

    c. the thymus of adult meadowlarks increased in wet weight during May and June, 1975 (Preston and Lewis, 1978).

2. However, a substantial number of *adults* still possess regressed thymuses in May and June. This tentative conclusion is supported by the following histological observations:

TABLE 8.4. PRELIMINARY INFORMATION CONCERNING MONTHLY CHANGES IN THE HISTOLOGY OF THE THYMUS GLAND OF WESTERN MEADOWLARKS (*Sturnella neglecta*)

Year	Month[1]	n	Average Number of Thymic Lobes Evaluated Per Bird	Mitotic Activity of Thymus[2]	Degree of Differentiation of Thymus into Cortex and Medulla[3]	X-Sectional Area (cm^2)			C/M Ratio[4, 5]
						Cortex	Medulla	Total	
1975	May	3	1.7	1.0	1.0	1.24	1.69	2.96	1.01
	Jun	1	2.0	1.0	1.0	0.63	0.68	1.31	0.85
1976	Apr	1	1.0	1.0	2.0	--	--	6.08	--
	May	5	3.4	1.2	1.2	1.34 (3)	0.50 (3)	1.63	1.37 (3)
	Jun	--	---	---	---	--	--	--	--
	Jul	3	5.0	1.7	1.0	1.22	1.48	2.70	1.31
	Aug	8	7.1	1.5	1.0	2.52	1.96	4.47	1.41
	Sep	5	4.8	1.0	1.0	2.10	1.76	4.87	0.90
1977	Apr	7	2.9	1.0	2.0	--	--	1.13	--
	May	49	4.6	1.2	1.7	0.72 (19)	1.36 (19)	1.48	0.68 (19)
	Jun	8	5.6	1.1	1.6	1.12 (3)	2.16 (3)	2.53	0.56 (3)

[1] The monthly values are averages computed from birds of both sexes and two age categories (juveniles and adults).

[2] The mitotic activity of each lobe of the thymus was estimated on a scale of 0 to 5 (none to very high).

[3] Each lobe of the thymus was assigned the number "1" if it had a distinct cortex and medulla; the number "2" if it lacked a distinct cortex and medulla.

[4] Values in parentheses in the table are numbers of observations which differ from the "n" value for the row.

[5] C/M Ratio = the ratio of the cross-sectional areas of the cortex (C) and medulla (M).

TABLE 8.5. PRELIMINARY INFORMATION CONCERNING MONTHLY CHANGES IN HASSAL'S CORPUSCLES IN THE THYMUS GLAND OF WESTERN MEADOWLARKS (*Sturnella neglecta*)

Year	Month[1]	n	Number of Hassal's Corpuscles Per Section[2]			Density of Hassal's Corpuscles (No./cm^2)	Number of Cysts Per Late Hassal's Corpuscle	Largest Diameter of Cysts (µm)
			Early Hassal's Corpuscles	Late Hassal's Corpuscles	Total Hassal's Corpuscles			
1975	May	3	4.7	17.3	22.0	9.9	2.9	25.7
	Jun	1	6.5	16.0	22.5	20.0	3.0	28.2
1976	Apr	1	9.0	19.0	28.0	4.6	3.0	25.8
	May	5	4.6	10.2	14.8	9.9	2.0	25.6
	Jun	-	--	--	--	--	--	--
	Jul	3	9.7	19.8	23.5	9.1	2.7	32.6
	Aug	8	21.1	26.8	47.8	12.7	3.0	54.3
	Sep	5	25.3	26.4	51.7	13.3	2.4	44.0
1977	Apr	7	11.6	11.5	23.1	23.2	2.7	21.9
	May	49	19.7	18.7	38.4	28.3	2.9	42.3
	Jun	8	42.1	25.0	67.1	28.9	2.8	43.1

[1] The monthly values are averages computed from birds of both sexes and two age categories (juveniles and adults).

[2] Criteria used to distinguish early and late Hassal's corpuscles are modified from Bacchus and Kendall (1975. Histological Changes Associated with Enlargement and Regression of the Thymus Glands of the Red-billed Quelea *Quelea quelea* L. (Ploceidae: Weaver-birds). *Phil. Trans. Roy. Soc. London B, Biol. Sci.*, 273:65-78) and presented in detail in the text of this paper.

TABLE 8.6. PRELIMINARY INFORMATION CONCERNING AGE- AND SEX-RELATED DIFFERENCES EACH MONTH IN THE HISTOLOGY OF THE THYMUS GLAND OF WESTERN MEADOWLARKS (*Sturnella neglecta*)

Sampling Period[1]	Age-Sex[2]	n	Average Number of Thymic Lobes Evaluated Per Bird	Mitotic Activity of Thymus[3]	Degree of Differentiation of Thymus into Cortex and Medulla[4]	X-Sectional Area (cm^2)			C/M Ratio[5]
						Cortex	Medulla	Total	
Apr 1976–1977	Ad - M	8	2.6	1.0	2.0	--	--	--	0.00
May 1975–1977	Ad - M	27	4.3	1.1	1.6	0.57 (12)	1.15 (12)	1.36	0.66 (12)
	Ad - F	30	4.4	1.1	1.6	1.13 (13)	1.43 (13)	1.76	0.94 (13)
Jun 1975–1977	Ad - M	6	4.5	1.0	1.7	1.10 (2)	1.97 (2)	2.38	0.69 (2)
	Ad - F	3	6.7	1.3	1.3	0.90 (2)	1.61 (2)	2.41	0.58 (2)
Jul 1976	Ad - F	1	3.0	2.0	1.0	0.77	0.66	1.43	1.34
	Ju - M	2	6.0	1.5	1.0	1.44	1.89	3.33	1.32
Aug 1976	Ad - M	2	7.0	2.0	1.0	1.03	0.99	2.01	1.08
	Ad - F	1	13.0	1.0	1.0	1.65	1.39	3.04	1.57
	Ju - M	1	8.0	3.0	1.0	4.98	3.78	8.76	1.64
	Ju - F	4	5.5	1.0	1.0	2.87	2.13	4.99	1.47
Sep 1976	Ad - M	1	2.0	1.0	1.0	1.45	1.34	2.80	1.20
	Ad - F	2	4.5	1.0	1.0	1.57	2.57	4.14	0.86
	Ju - F	2	6.5	1.0	1.0	2.95	3.69	6.64	0.80

---

[1] Data for each age-sex group collected in 1975, or 1976, through 1977 were lumped in Apr through Jun. Data for Jul through Aug 1977 are not yet available. Values in parentheses in the table are numbers of observations which differ from the "n" value for the row.

[2] Ad = adult; Ju = juvenile; M = male; F = female.

[3] The mitotic activity of each lobe of the thymus was estimated on a scale of 0 to 5 (none to very high).

[4] Each lobe of the thymus was assigned the number "1" if it had a distinct cortex and medulla; the number "2" if it lacked a distinct cortex and medulla.

[5] C/M Ratio = the ratio of the cross-sectional areas of the cortex (C) and medulla (M).

TABLE 8.7. PRELIMINARY INFORMATION CONCERNING AGE- AND SEX-RELATED DIFFERENCES EACH MONTH IN HASSAL'S CORPUSCLES WITHIN THE THYMUS GLAND OF WESTERN MEADOWLARKS (*Sturnella neglecta*)

Sampling Period[1]	Age-Sex[2]	n	Number of Hassal's Corpuscles Per Section[3]			Density of Hassal's Corpuscles (No./cm^2)	Number of Cysts Per Late Hassal's Corpuscle	Largest Diameter of Cysts (μm)
			Early Hassal's Corpuscles	Late Hassal's Corpuscles	Total Hassal's Corpuscles			
Apr 1976-1977	Ad - M	8	11.1	12.4	23.7	21.0	2.8	22.4
May 1975-1977	Ad - M	27	16.1	14.4	30.5	25.0	2.8	33.6
	Ad - F	30	19.0	21.0	40.0	26.3	2.7	45.6
Jun 1975-1977	Ad - M	6	37.3	21.6	58.9	26.1	2.7	36.1
	Ad - F	3	39.8	28.7	68.6	31.3	3.0	52.2
Jul 1976	Ad - F	1	5.0	11.7	16.7	11.5	2.0	94.8
	Ju - M	2	12.1	14.8	26.9	7.9	3.0	31.5
Aug 1976	Ad - M	2	10.6	15.5	26.0	14.7	3.0	48.2
	Ad - F	1	15.3	29.1	44.4	15.6	3.0	64.6
	Ju - M	1	34.4	39.1	73.5	8.5	2.0	53.5
	Ju - F	4	24.5	28.7	52.5	12.1	3.3	55.1
Sep 1976	Ad - M	1	13.5	12.5	26.0	16.4	3.0	19.2
	Ad - F	2	20.5	13.4	33.8	9.9	2.0	28.0
	Ju - F	2	36.1	46.4	82.5	15.0	2.5	77.5

[1] Data for each age-sex group collected in 1975, or 1976, through 1977 were lumped in Apr through Jun. Data for Jul through Aug 1977 are not yet available.

[2] Ad = adult; Ju = juvenile; M = male; F = female.

[3] Criteria used to distinguish early and late Hassal's corpuscles are modified from Bacchus and Kendall (1975. Histological Changes Associated with Enlargement and Regression of the Thymus Glands of the Red-billed Quelea *Quelea quelea* L. (Ploceidae: Weaver-birds). *Phil. Trans. Roy. Soc. London B, Biol. Sci.*, 273:65-78) and presented in detail in the text of this paper.

   a. the density of Hassal's corpuscles (total number per unit area of adult gland) is substantially higher during the April-June period than during the July-September period (Table 8.7)

   b. differentiation of the cortex had not begun in April and was not complete (i.e., average values do not equal 2.0) in all adults in May and June (Table 8.6).

3. The thymus of *adult* meadowlarks remains enlarged and active between July and at least September (when collections stopped). This tentative conclusion is supported by the following histological observations:

   a. a cortex was present in the thymuses of all adult meadowlarks between July and September (Tables 8.4 and 8.6)

   b. the density of Hassal's corpuscles was lower during the July-September period than during April, May, and June (Table 8.7).

4. Changes in the cross-sectional areas of the cortex and medulla of *adult* glands suggest that this may be an easily measured and accurate index of thymic activity since the C/M ratio consistently exceeds 1.0 (i.e., the area of the cortex is greater than that of the medulla) only when the adult gland is fully enlarged (Table 8.6).

5. *Juvenile* meadowlarks have larger and more active thymus glands than molting adults. This tentative conclusion is supported by the following observations:

   a. the mitotic activity of the thymuses of juvenile birds was frequently low during the July-September period, suggesting that the glands were already fully enlarged; since adult glands also appeared to be fully developed at this time (Table 8.6 and conclusion no. 3 above), differences in juvenile and adult glands during this period are between glands at the same stage of development

   b. the cross-sectional areas of cortex, medulla, and whole thymus were considerably higher in juvenile birds than in adults collected during July, August, and September (Table 8.6)

   c. the thymus glands of juvenile meadowlarks weighed more than those of adults during July and August, 1975 (Preston and Lewis, 1978).

More specific comments about how the thymus and periods of reproduction and molt are related will be made after we have the opportunity to examine all of the thymuses.

CONCLUSIONS

The thyroid gland of the Western Meadowlark consists of secretory follicles which are laden with colloid and lined with a simple epithelium. Connective and vascular tissues occur between the follicles.

Five histological measures of thyroid activity were used to evaluate the gland. Nuclear diameter and epithelial height appear to be the most sensitive indicators of small changes in glandular function. Four histometric criteria (diameter and colloid content of the follicles; diameter of epithelial nuclei; and height of the follicular epithelium) indicate that the thyroid gland of *adult* meadowlarks is relatively inactive during the breeding season, becomes increasingly active during subsequent gonadal regression, and is highly active during the period of postnuptial molt which follows. No sexual differences exist in thyroid glands of adult or juvenile meadowlarks during reproductive and molt periods. Age-specific differences occur only in June when young birds are fledging. At this time, the juvenile gland is more active than the adult gland. These conclusions are based on the examination of thyroid glands from 363 meadowlarks collected during two field seasons (1976-1977).

The thymus gland of the Western Meadowlark consists of a series of independent lobes closely associated with the jugular veins and the trachea. The individual lobes of each meadowlark are histologically similar to each other. The parenchyma of the gland consists of an inner medulla and an outer cortex, or of a medulla only. If a cortex is present, it is extremely dense and cellular. Small lymphocytes account for 70-90% of the cells in this region. The rest are reticular cells, medium- and large-sized lymphocytes, erythroblasts, erythrocytes, plasma cells, fibroblasts, and mitotic and pycnotic elements. Large spaces are also present. They are commonly empty, but may contain laminated concretions and droplets of an homogenous fluid. A network of small capillaries vascularizes the cortex.

The medulla is less dense than the cortex, primarily because it contains fewer cells. However, the same cell types occur in both medulla and cortex. Reticular cells in the medulla are commonly vacuolated. Many large extracellular vacuoles and cysts are also present. They frequently contain eosinophilic fluid. The cysts are frequently ciliated. Numerous Hassal's corpuscles of several kinds (early, late, laminated) and scattered individual myoid cells also populate the medulla. Capillaries, veins, and arterioles vascularize this region.

A preliminary survey suggests that the thymus gland of *adult* meadowlarks recrudesces during the reproductive season and is fully developed and active when the postnuptial molt begins. It remains enlarged and functional during the molt period. Juvenile birds have larger and more active thymus glands than molting adults. These tentative conclusions are based on examination of thymus glands from 90 meadowlarks collected during three field seasons (1975-1977).

REFERENCES

Anderson, W.L. 1970. Seasonal Changes in Thymus Weights in Ring-necked Pheasants. Condor, 72(2):205-208.

Assenmacher, I. 1973. The Peripheral Endocrine Glands. In: Avian Biology, Vol. III, D.S. Farner and J.R. King, eds. Academic Press Inc., New York. pp. 183-286.

Bacchus, S., and M.D. Kendall. 1975. Histological Changes Associated with Enlargement and Regression of the Thymus Glands of the Red-billed Quelea *Quelea quelea* L. (Ploceidae: Weaver-birds). Phil. Trans. Roy. Soc. London B, Biol. Sci., 273(923):65-78.

Berthold, P. 1975. Migration: Control and Metabolic Physiology. In: Avian Biology, Vol. V, D.S. Farner and J.R. King, eds. Academic Press Inc., New York. pp. 77-128.

Blau, J.N. 1967. The Dynamic Behavior of Hassal's Corpuscles and the Transport of Particulate Matter in the Thymus of the Guinea-Pig. Immunology, 13(3):281-292.

Bloom, W., and D.W. Fawcett, eds. 1975. A Textbook of Histology, 10th Ed. W.B. Saunders Co., Philadelphia, Pennsylvania. 1033 pp.

Burger, J.W. 1938. Cyclic Changes in the Thyroid and Adrenal Cortex of the Male Starling, *Sturnus vulgaris,* and their Relation to the Sexual Cycle. Amer. Nat., 72(743):562-570.

Cooper, M.D., D.A. Raymond, M. Peterson, A. South, and R. Good. 1966. The Functions of the Thymus System and the Bursa System in the Chicken. J. Exp. Med., 123(1):75-102.

Davis, J., and B.S. Davis. 1954. The Annual Gonad and Thyroid Cycles of the English Sparrow in Southern California. Condor, 56(4):328-345.

Duncan, D.B. 1955. Multiple Range and Multiple F Tests. Biometrics, 11(1): 1-42.

Erpino, M.J. 1968. Aspects of Thyroid Histology in Black-billed Magpies. Auk, 85(3):397-403.

Falconer, I.R. 1971. The Thyroid Glands. In: Physiology and Biochemistry of the Domestic Fowl, Vol. 1, D.J. Bell and B.M. Freeman, eds. Academic Press Inc., New York. pp. 459-472.

Gelineo, S. 1955. Temperature d'Adaptation et Production de Chaleur chez les Oiseaux de Petite Taille. Arch. Sci. Physiol., 9:225-243.

George, J.C., and D.V. Naik. 1964. Cyclic Changes in the Thyroid of the Migratory Starling, *Sturnus roseus* (Linnaeus). Pavo, 2:37-49.

Hahn, D.W., T. Ishibashi, and C.W. Turner. 1966. Alteration of Thyroid Hormone Secretion Rate in Fowls Changed from a Cold to a Warm Environment. Poultry Sci., 45(1):31-33.

Höcker, W. 1967. Über lokale bzw. lokalisierte Trijodthyronin-wirkungen auf das Wachstum aktivierter Federkeime. Naturwissenschaften, 54:207.

Hodges, R.D. 1974. The Histology of the Fowl. Academic Press Inc., New York. 648 pp.

Höhn, E.O. 1949. Seasonal Changes in the Thyroid Gland and Effects of Thyroidectomy in the Mallard, in Relation to Molt. Amer. J. Physiol., 158(3):337-344.

_______. 1950. Physiology of the Thyroid Gland in Birds: A Review. Ibis, 92(4):464-473.

_______. 1956. Seasonal Recrudescence of the Thymus in Adult Birds. Can. J. Biochem. Physiol., 34(1):90-101.

_______. 1961. Endocrine Glands, Thymus and Pineal Body. In: Biology and Comparative Physiology of Birds, Vol. 2, A.J. Marshall, ed. Academic Press Inc., New York. pp. 87-114.

Höhn, E.O., and C.E. Braun. 1977. Seasonal Thyroid Gland Histophysiology and Weight in White-tailed Ptarmigan. Auk, 94(3):544-551.

Kendall, M.D. 1975. Sizes and Numbers of Nuclei in the Cortex of Thymus Glands of Red-billed Weavers *Quelea quelea*. Cell Tissue Res., 164(2): 233-249.

Kramer, C.Y. 1956. Extension of Multiple Range Tests to Group Means with Unequal Numbers of Replications. Biometrics, 12(2):307-310.

Lewis, R.A., N.R. Glass, and A.S. Lefohn, eds. 1975. The Bioenvironmental Impact of a Coal-Fired Power Plant, 2nd Interim Report, Colstrip, Montana. EPA-600/3-76-013, U.S. Environmental Protection Agency, Corvallis, Oregon. 315 pp.

Ljunggren, L. 1968. Seasonal Studies of Wood Pigeon Populations. I. Body Weight, Feeding Habits, Liver and Thyroid Activity. Viltrevy, Swedish Wildlife, Uppsala, 5(9):435-504.

Merkel, F.W. 1938. Zur Physiologie der Zugenruhe bei Vögeln. Ber. Ver. Schles. Ornithol., 25:1-72.

Moticka, E.J. 1975. Development of Immunological Competence in Chickens. Amer. Zool., 15(1):135-146.

Payne, L.N. 1971. The Lymphoid System. In: Physiology and Biochemistry of the Domestic Fowl, Vol. 2, D.J. Bell and B.M. Freeman, eds. Academic Press Inc., New York. pp. 985-1037.

Payne, R.B. 1972. Mechanisms and Control of Molt. In: Avian Biology, Vol. II, D.S. Farner and J.R. King, eds. Academic Press Inc., New York. pp. 103-155.

Payne, R.B., and M. Landolt. 1970. Thyroid Histology of Tricolored Blackbirds (*Agelaius tricolor*) in the Annual Cycle, Breeding, and Molt. Condor, 72(4):445-451.

Preston, E.M., and R.A. Lewis, eds. 1978. The Bioenvironmental Impact of a Coal-Fired Power Plant, 3rd Interim Report, Colstrip, Montana. EPA-600/3-78-021, U.S. Environmental Protection Agency, Corvallis, Oregon. 521 pp.

Putzig, P. 1938. Der Frühwegzug des Kiebitzes. J. Ornithol., 86:123-165.

Raitt, R.J. 1968. Annual Cycle of Adrenal and Thyroid Glands in Gambel Quail of Southern New Mexico. Condor, 70(4):366-372.

Raviola, E. 1975*a*. The immune System. In: A Textbook of Histology, 10th Ed., W. Bloom and D.W. Fawcett, eds. W.B. Saunders Co., Philadelphia, Pennsylvania. pp. 427-456.

_______. 1975*b*. Thymus. In: A Textbook of Histology, 10th Ed., W. Bloom and D.W. Fawcett, eds. W.B. Saunders Co., Philadelphia, Pennsylvania. pp. 457-470.

Ringer, R.K. 1975. Thyroids. In: Avian Physiology, 3rd Ed., P.D. Sturkie, ed. Springer-Verlag, New York. pp. 348-358.

Saatman, R.R., and A. van Tienhoven. 1964. Effect of Thyroxin on Assay of Thyroid-stimulating Hormone. Amer. J. Physiol., 206(1):89-92.

Steel, R.G.D., and J.H. Torrie. 1960. Principles and Procedures of Statistics with Special Reference to the Biological Sciences. McGraw-Hill Book Co., Inc., New York. 481 pp.

Stickel, W.H. 1975. Some Effects of Pollutants in Terrestrial Ecosystems. In: Ecological Toxicology Research, A.D. McIntyre and C.F. Mills, eds. Plenum Press, New York. pp. 25-74.

Truhaut, R. 1975. Ecotoxicology--A New Branch of Toxicology: A General Survey of its Aims, Methods, and Prospects. In: Ecological Toxicology Research, A.D. McIntyre and C.F. Mills, eds. Plenum Press, New York. pp. 3-23.

Voitkevich, A.A. 1966. The Feathers and Plumage of Birds. October House, New York.

Von Faber, H. 1967. Die Beziehungen von Kerngrösse und histologischem Bild zur Thyroxinsekretionsrate der Schilddrüse. Zool. Anz., 30 Suppl.: 172-175.

Ward, P., and D. D'Cruz. 1968. Seasonal Changes in the Thymus Gland of a Tropical Bird. Ibis, 110(2):203-205.

Ward, P., and M.D. Kendall. 1975. Morphological Changes in the Thymus of Young and Adult Red-billed Queleas *Quelea quelea* (Aves). Phil. Trans. Roy. Soc. London B, Biol. Sci., 273(923):55-64.

Wilson, A.C., and D.S. Farner. 1960. The Annual Cycle of Thyroid Activity in White-crowned Sparrows of Eastern Washington. Condor, 62(6):414-425.

THE EFFECTS OF CONTROLLED FIELD EXPOSURES TO SO_2 ON BIOLOGICAL SYSTEMS OF THE NORTHERN GREAT PLAINS

SECTION 9

TEMPORAL VARIATION IN SO_2 CONCENTRATION ON ZAPS

J. J. Lee, E. M. Preston, and D. B. Weber

ABSTRACT

Sulfur dioxide concentrations on the ZAPS plots were monitored with a real-time flame photometric sulfur gas anlyzer during the 1976 and 1977 field seasons. By monitoring multiple locations within same plots, intra-plot comparisons were possible. By monitoring comparable locations on all plots, inter-plot comparisons were possible. Sources of uncertainty in the SO_2 measurements are evaluated. SO_2 concentrations on the ZAPS plots show substantial inter-seasonal and intra-seasonal variation. Concentrations are generally higher at night than during the day, but otherwise patterns of intra-seasonal variability are not consistent between ZAPS plots or between years. Intra-season variation is greatest on high treatment plots and least on Control plots. Seasonal frequencies of SO_2 concentrations are approximately log-normally distributed and show reasonably good separation in fumigation histories of the treatment plots. Though median SO_2 concentrations on the Control (A) plots are small, these plots are subject to short-term acute fumigations due to drift from other plots. SO_2 concentrations are strongly correlated with the inverse of wind speed and slightly correlated with solar radiation, temperature-stability class, and relative humidity.

INTRODUCTION

Each Zonal Air Pollution System (ZAPS) study site consists of four 0.5 hectare (1 1/4 acre) treatment plots located along a line with 61 m intervening buffer zones to reduce interference between plots (see Lee and Lewis, 1978). Sulfur dioxide (SO_2) concentrations are continually measured at various standard locations on ZAPS I and ZAPS II (Figure 9.1) throughout the growing season. While not all locations on all plots are monitored, location _c_ is used on all plots, providing a basis for inter-plot comparisons. Use of multiple locations within some plots allows intra-plot comparisons.

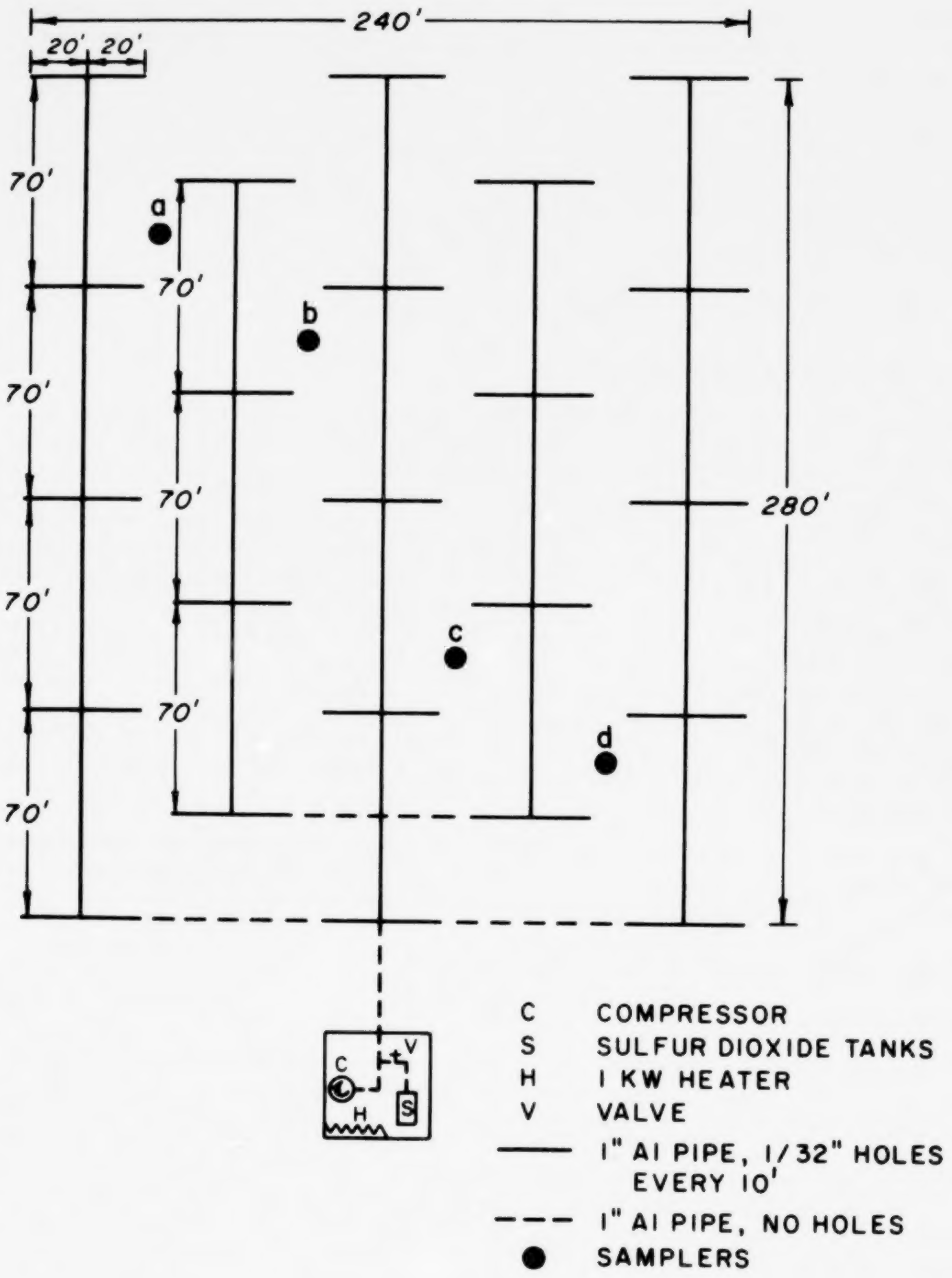

240'
20'
20'
70'
70'
70'
70'
70'
70'
70'
70'
a
b
c
d
280'
C
V
H
S
C COMPRESSOR
S SULFUR DIOXIDE TANKS
H 1 KW HEATER
V VALVE
1" Al PIPE, 1/32" HOLES EVERY 10'
1" Al PIPE, NO HOLES
● SAMPLERS

Figure 9.1. Standard probe locations.

SO_2 flow rates from the SO_2 tanks to the delivery pipes were essentially the same in 1976 and 1977 as in 1975, namely 0, 0.7, 1.7, and 3.3 standard liters per minute on plots A, B, C, and D, respectively. Concentrations varied stochastically according to micrometeorological conditions.

In this section, we describe data collection and synthesis procedures and discuss sources of uncertainty in the SO_2 measurements. We present averages and peaks observed during the 1976 and 1977 field seasons, as well as the results of investigating the variation of SO_2 concentrations with micrometeorological variables.

MATERIALS AND METHODS

Design of the Monitoring System

Figure 9.2 is a schematic diagram of the SO_2 monitoring system. SO_2 concentrations on the plots were monitored by recording the output from a Meloy model SA160-2 flame photometric sulfur gas analyzer operating in logarithmic mode. Samples were continuously drawn through teflon tubing extending to the monitoring locations by a time-share device (Adgo Co.). The continuous streams travel to the solenoids where they are diverted either to the sulfur gas analyzer via the sample manifold or out the exhaust via the common manifold. A stepping switch in the time share device provides 7.5 minutes of continuous electrical energy per hour to each of the solenoids sequentially. During this 7.5 minutes, the energized solenoid directs the incoming sample gas into the sample manifold while the remaining seven non-energized solenoids divert sample gas through the common manifold to the exhaust. Gas flow through the line being sampled is produced by the sulfur gas analyzer while gas flow through the remaining seven lines is maintained by the common manifold pump.

The sulfur gas analyzer was calibrated against NBS-SRM 1627 permeation tubes using either a Model 303 gas mixing system (Analytical Instrument Development, Inc.) or a Model 330 Dynacalibrator (Metronics).

Sources of Uncertainty in SO_2 Measurements

Response Characteristics of the System

Several factors affect the time required for the monitoring system to respond to change in SO_2 concentrations on the sample plots. First, as sample line length increases, the time required for the sample to reach the analyzer increases. During 1975, 1976, and 1977, sample line length varied from 98 meters to 299 meters. Gas streams produced by the common manifold pump take less than 5 minutes to reach the time-share device. Secondly, teflon sample lines with two different wall thicknesses were used. The thinner tubing has a longer response time due to larger cross-sectional area for transport through the lines. Thirdly, the system responds more quickly to higher sulfur levels than to low ones. Fourthly, response time is influenced by the degree of SO_2 conditioning of the teflon sample lines. Lines which have been conditioned longer respond more quickly than those conditioned for a shorter period.

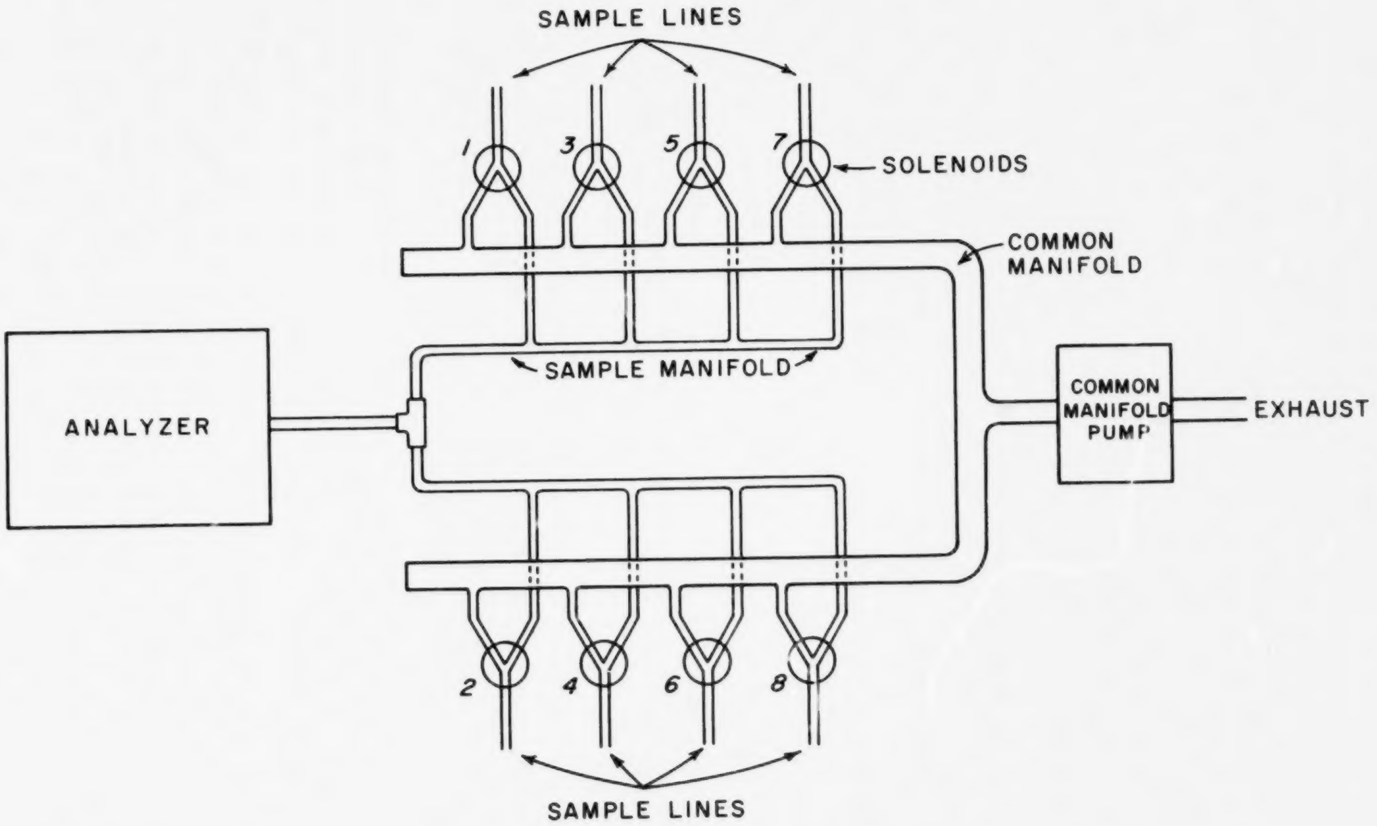

SAMPLE LINES
1
3
5
7
SOLENOIDS
COMMON MANIFOLD
SAMPLE MANIFOLD
ANALYZER
COMMON MANIFOLD PUMP
EXHAUST
2
4
6
8
SAMPLE LINES

Figure 9.2. Schematic diagram of the sulfur analyzer hookup with sample lines from the field presently in operation at the ZAPS study site.

Therefore, response time is probably somewhat longer earlier in the season than later in the season. Since response time is influenced by several factors in ways difficult to quantify, no comprehensive scheme of response characteristics is attempted. It can be said, however, that fine time detail (instantaneous peaks) are not measurable by the system. Line lengths were equalized at the start of the 1978 field season. This should make response characteristics at different measuring points somewhat more similar.

Non-linearity of Calibration Curves

Calibration curves relating analyzer response to the logarithm of SO_2 concentration typically became non-linear at SO_2 concentrations below roughly 2 pphm. Available calibration equipment did not permit determination of the precise nature of the non-linearity. Since linear extrapolation on these calibration curves to zero analyzer response lead to estimated SO_2 concentrations equal to or greater than 0.9 pphm, it is highly probable that linear extrapolation from calibration curves leads to an overestimate of true SO_2 levels in the region between 0 pphm and 2 pphm. The true value, in nearly all cases, will lie between 0.1 pphm (probable background concentrations) and the value estimated from the calibration curve. These values were used to specify upper and lower bounds for any SO_2 concentration falling in the questionable region of the calibration curves.

Carbon Dioxide Interference During Calibration With the Metronics Calibrator

It has recently been shown that carbon dioxide concentration significantly affects the response characteristics of FPD sulfur gas analyzers (Weber *et al.*, 1978). Carbon dioxide depresses response by a factor log proportional to CO_2 concentration (Weber *et al.*, 1978).

Two calibration devices have been used on the ZAPS monitors. The first, manufactured by Analytical Instrument Development Inc., was used on ZAPS I previous to May 25, 1977 and on ZAPS II previous to May 27, 1977. This calibrator uses a tank of compressed air as a carrier for creating the calibration gas. Tank air contains CO_2 levels similar to ambient levels.

A second device, which uses the air inside the monitoring shed as a carrier for the calibration gas (Metronics) has been used since June 1977. In the monitoring shed with one person performing an SO_2 calibration, we measured CO_2 levels of roughly 485 ppm. Ambient CO_2 concentration is roughly 330 ppm.

The degree of CO_2 interference during past calibrations is impossible to quantify precisely, since CO_2 levels present in the monitoring shed during previous calibrations are not precisely known. Upper bounds for the CO_2 interference effect have been estimated assuming a CO_2 level of 485 ppm during all calibrations using the Metronics Dynacalibrator. Calibration curves can then be corrected for this interference using relationships defined by Weber *et al.* (1978). The problem was solved during the 1978 field season by attaching Metronics' pump inlets to tubing running to external air.

Dilution of Sample Due to Leaks in Sample Lines

Any leaks in sample lines will cause sample dilution and cause errors in estimates of sample SO_2 levels. Sample lines were walked regularly to check by visual inspection for holes caused by small mammals, researchers, etc. During 1978, we checked for leaks in sample lines with vacuum measurements. This procedure demonstrated that small leaks could go undetected by the previously utilized visual inspection method. Thus, small errors could have been introduced through undetected leaks in sample lines during 1976 and 1977. However, sample lines were checked regularly and carefully by visual inspection and it is unlikely that substantial SO_2 measurement errors were introduced by sample line leaks.

Uncertainties Introduced During Data Transcription

Response of the sulfur gas analyzers to SO_2 is recorded continuously on analog strip charts. Comparisons of data obtained by direct readout from a digital voltmeter with data transcribed from strip charts indicate that analog data can be converted to digital data with error limits of ± 3%.

The average chart value (millivolts) used is that which balances curve length; *i.e.*, total curve length above this point equals total curve length below this point for the 7.5 minute sample interval. Since the SO_2 analyzer output is linear in the logarithm of the concentration, this estimates the average logarithm. Assuming log-normality, this is the best estimate of the median logarithm, and thus of the median rather than average concentration. The adequacy of this procedure was tested by short term, intensive study. Several points were recorded for each 7.5 minute interval for ZAPS I from April 16 through April 23, 1977. For each interval, the average and median of these points was compared to the "equal length" value. The results (Table 9.1a) indicate that the three procedures are equivalent, and that the "equal length" measurement adequately estimates the average scale reading.

TABLE 9.1a. RELATIONS BETWEEN EQUAL CURVE LENGTH, MEDIAN, AND AVERAGE SCALE READINGS, ZAPS II, PROBE Dd, 4/16/76 - 4/23/76

y = average scale reading

x_1 = "equal curve length" scale reading

x_2 = median scale reading

Regression: $Ey = ax_i$

x_i	a	r	r^2
x_1	.9947	.9990	.9981
x_2	1.0013	.9989	.9978

Uncertainty Introduced by Using 7.5 Minute Median, Rather Then Average, Concentrations Obtained from the Above Intensive Study

A regression of average concentrations with the median concentrations (Table 9.1b) indicates that the 7.5 minute median is approximately 2% less than the corresponding 7.5 minute average. Since, for log-normal distributions,

$$\text{Mean} = (\text{median})\ e^{\frac{1}{2}\sigma^2},$$

where $\sigma = \ln$ (SGD),

this implies a SGD of 1.22 for time intervals of less than 7.5 minutes. This low value is close to the theoretical minimum SGD of 1.0, and reflects the high correlation between concentrations over short time intervals.

TABLE 9.1b. COMPARISON OF 7.5 min MEDIAN AND AVERAGE SO_2 CONCENTRATIONS, ZAPS II, PROBE Dd, 4/16/76 - 4/23/76

Y = Average SO_2 Concentration
X = Median SO_2 Concentration
$Ey = Ax$
$A = 1.021 \pm .003$
$r = .9996$
$r^2 = .9991$

Variable Vacuums Between Sample Lines

As previously noted during 1976 and 1977, sample line lengths to different monitoring positions varied. As the analyzer pump attempts to draw sample air through the line a vacuum is created whose magnitude is proportional to line length. The magnitude of this vacuum influences analyzer response. Since every sample line had a unique length, each also had a unique calibration curve. However, a common calibration curve for all sample lines was assumed in estimating SO_2 levels. Measurements of the effect on analyzer response of vacuums generated by different line lengths demonstrated that errors introduced by assuming a common calibration curve for each line are generally less than 3%.

Synthesis Procedures

At each ZAPS site, a total of eight sample lines feed to a time-share device. These lines are selected sequentially, so that any one line is connected to the SO_2 analyzer for a single 7.5 minute interval each hour. The output from the analyzer, which is linear in the logarithm of the concentration, is continuously recorded on a strip chart. One of the lines passes through a zero-SO_2 filter, providing a check for drift. The other seven probes are located on the plots, with at least one probe per plot.

The 7.5 minute averages for each probe are used directly to estimate weekly, monthly and seaonal geometric means (GM), standard geometric deviations (SGD), and arithmetic means (AM). To estimate the sequence of 1-hr, 3-hr, and 24-hr averages, we need continuous readings for each probe. These are estimated using the concentrations for each 7.5 minute interval concentration at the probe being monitored. The synthesis procedure thus consists of five major steps:

(1) Obtain average SO_2 concentrations for each 7.5 minute interval for probe being monitored.

(2) Use (1) to estimate concentrations at standard probe.

(3) Calculate hourly and longer average concentrations at standard probe.

(4) Use (3) to estimate average concentrations at other probes.

(5) Form weekly GM's and SGD's of the 1-hr, 3-hr, and 24-hr averages; also, keep account of violations of Federal and State air quality standards.

A generalized flow chart for the synthesizing computer program is shown in Figure 9.3.

The first major step consists of reading average values for each interval from the strip chart, subtracting the zero-filter reading (usually zero) to allow for drift, and entering this value into the calibration equations.

Since errors in SO_2 concentration estimates caused by CO_2 interference and non-linearity of calibration curves cannot be expressed as simple percentages of estimated concentrations, each strip chart average was entered into two calibration equations. In one, the HIGH run, the calibration equation was adjusted to yield a maximum estimate that could result from from the above sources of uncertainty. In the other, the LOW run, the calibration equation was adjusted to yield the corresponding minimum estimate. Summary statistics were computed for both the HIGH run and LOW run. The range of values presented in RESULTS AND DISCUSSION brackets the true values.

The second major step is accomplished by scaling all data to the same geometric mean. After all data for a week are read, the GM's for all probes for that week are calculated. The concentration for each 7.5 minute interval for the standard probe, s, is estimated by multiplying the measured concentration at probe i by the ratio of weekly GM's,

$$(1)\quad C_s\ (\text{est}) = C_i\ (\text{meas})\ \frac{GM_s}{GM_i}\ .$$

This is equivalent to centering the normal distributions of logarithms at the same value. Earlier analysis of ZAPS data indicated that SO_2 concentrations at various locations (except for the control plot) varied similarly when

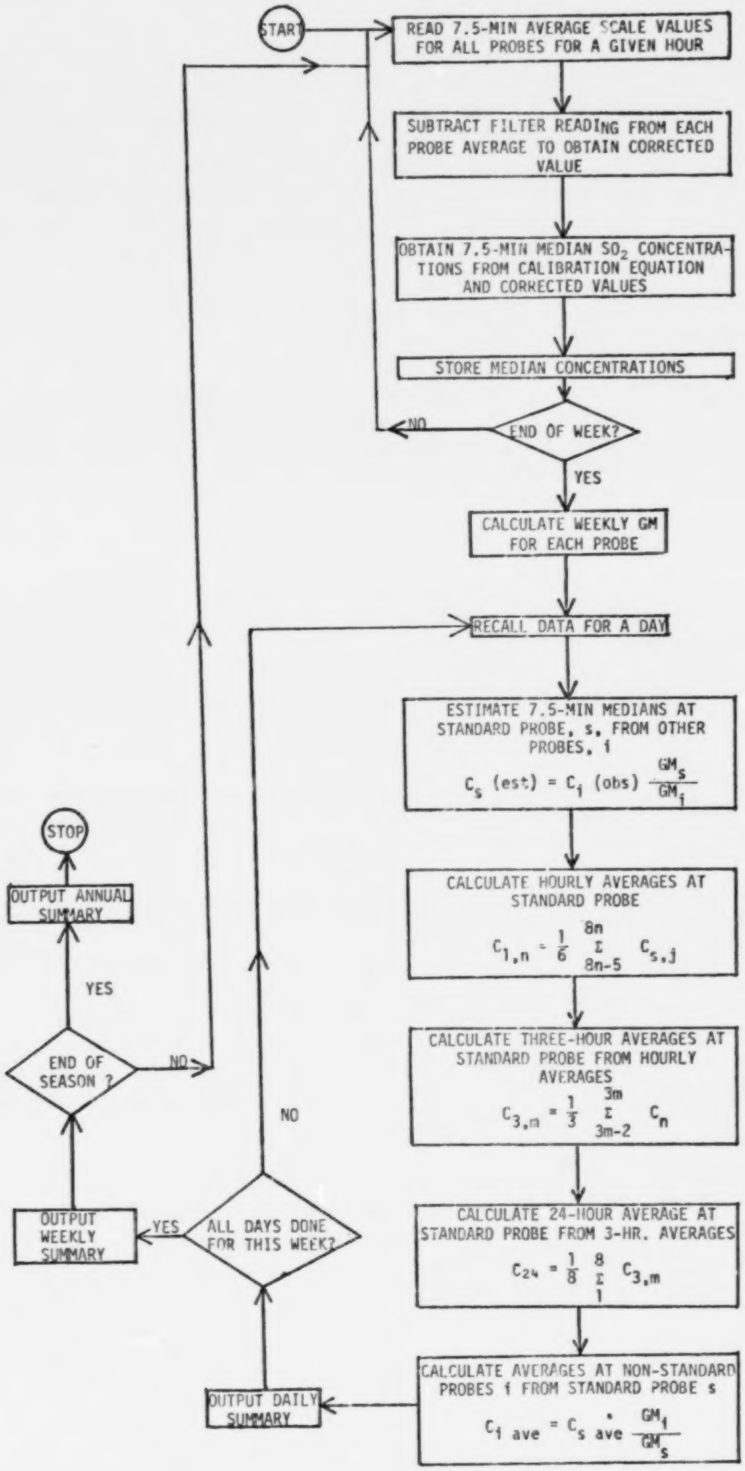

START
READ 7.5-MIN AVERAGE SCALE VALUES FOR ALL PROBES FOR A GIVEN HOUR
SUBTRACT FILTER READING FROM EACH PROBE AVERAGE TO OBTAIN CORRECTED VALUE
OBTAIN 7.5-MIN MEDIAN SO_2 CONCENTRATIONS FROM CALIBRATION EQUATION AND CORRECTED VALUES
STORE MEDIAN CONCENTRATIONS
END OF WEEK?
NO
YES
CALCULATE WEEKLY GM FOR EACH PROBE
RECALL DATA FOR A DAY
ESTIMATE 7.5-MIN MEDIANS AT STANDARD PROBE, s, FROM OTHER PROBES, i
C_s (est) = C_i (obs) $\frac{GM_s}{GM_i}$
CALCULATE HOURLY AVERAGES AT STANDARD PROBE
$C_{1,n} = \frac{1}{6} \sum_{8n-5}^{8n} C_{s,j}$
CALCULATE THREE-HOUR AVERAGES AT STANDARD PROBE FROM HOURLY AVERAGES
$C_{3,m} = \frac{1}{3} \sum_{3m-2}^{3m} C_n$
CALCULATE 24-HOUR AVERAGE AT STANDARD PROBE FROM 3-HR. AVERAGES
$C_{24} = \frac{1}{8} \sum_{1}^{8} C_{3,m}$
CALCULATE AVERAGES AT NON-STANDARD PROBES i FROM STANDARD PROBE s
$C_{i\ ave} = C_{s\ ave} \cdot \frac{GM_i}{GM_s}$
OUTPUT DAILY SUMMARY
ALL DAYS DONE FOR THIS WEEK?
NO
YES
OUTPUT WEEKLY SUMMARY
END OF SEASON ?
NO
YES
OUTPUT ANNUAL SUMMARY
STOP

Figure 9.3. Flow chart of data synthesis procedures.

normalized to the same GM (Lee and Lewis, 1978). This procedure is consistent with most models of pollutant dispersion, in which the concentration is estimated by multiplying a meteorological dispersion factor by a source strength scale factor (Turner, 1969).

The estimated 7.5 minute medians at the standard probe are combined into averages for non-overlapping time intervals of one hour and longer. For example, for a given day, all 7.5 minute medians between midnight and 1 a.m. are averaged, all those between 1 a.m. and 2 a.m., etc. This standard procedure (Larsen, 1971) yields 24 one-hour averages, 3 eight-hour averages, and 1 24-hour average per day. Averages for each non-standard probe, i, are estimated by multiplying the standard averages by the ratio $GM_i : GM_s$.

Concentrations for the plot receiving no direct SO_2 input (the Control plot, A) are excluded from this averaging process since these were usually below the detection threshold of the analyzer. For this probe, the 7.5 minute median is used to represent the entire hour, and 3-hour and 24-hour averages are based on these. Averages for this probe are thus much more sensitive to short term fluctuations, and consequently less reliable, than averages for other probes.

RESULTS AND DISCUSSION

Interseasonal Trends

The seasonal data summaries are given in Table 9.2, as are the 1-hour, 3-hour, and 24-hour peaks experienced during each season. The frequency of violations of Montana and Federal SO_2 standards during the growing seasons are also provided. Results are shown for both the High and Low run. Although SO_2 flow rates were not changed, both average and peak concentrations were generally higher in 1977 than in 1976 for ZAPS I. While median concentrations on ZAPS II were similar in 1976 and 1977, individual measurements were more variable in 1977.

Intraseasonal Trends

Sulfur dioxide concentrations varied considerably during the course of each growing season (Figure 9.4). The D-plots are particularly variable. Data for the 1977 field season, ZAPS I, Plot C seemed unusually high compared to those for 1975, 1976, and ZAPS II, C. Additional evidence presented in Section 10 (Figure 10.11) suggests that these values were artificially high. Values shown have been adjusted as described in Section 10. ZAPS II seems to have had a somewhat more uniform fumigation history than ZAPS I.

Frequency Distributions of SO_2 Concentrations on the Plots

Frequency distributions of SO_2 concentrations on the ZAPS plots are approximately log-normal (Figure 9.5). Deviation from log-normality appears to have increased in 1977 compared to 1976 on both ZAPS I and ZAPS II. Cumulative frequency distributions for different treatment plots are generally distinct while distributions for various points monitored within a treatment

TABLE 9.2a. ZAPS I SEASONAL SUMMARY, 1976
(pphm SO_2)

Probe Ac GM = .2-.9 SGD = 3.0, 1.5 Arithmetic Mean = .5-1.1

1-Hour Ave Exceeded 25 pphm	0 Times:	0 Percent
3-Hour Ave Exceeded 50 pphm	0 Times:	0 Percent
24-Hour Ave Exceeded 10 pphm	0 Times:	0 Percent
24-Hour Ave Exceeded 14 pphm	0 Times:	0 Percent

1-Hour Peak 13-13 3-Hour Peak 7.6-7.8 24-Hour Peak 2.4-3.1

Probe Bd GM = 1.4-1.9 SGD = 3.8, 2.2 Arithmetic Mean = 2.9-3.0

1-Hour Ave Exceeded 25 pphm	25-29 Times:	.7-.8 Percent
3-Hour Ave Exceeded 50 pphm	1-1 Times:	.1 Percent
24-Hour Ave Exceeded 10 pphm	2-2 Times:	1.3 Percent(a)
24-Hour Ave Exceeded 14 pphm	1-1 Times:	.6 Percent

1-Hour Peak 45-50 3-Hour Peak 26-32 24-Hour Peak 9.7-10.6

Probe Bc GM = 1.8-2.2 SGD = 3.6, 2.5 Arithmetic Mean = 3.9-4.0

1-Hour Ave Exceeded 25 pphm	22-35 Times:	.6-1.0 Percent
3-Hour Ave Exceeded 50 pphm	1-1 Times:	.1 Percent
24-Hour Ave Exceeded 10 pphm	1-2 Times:	.6-1.3 Percent(c)
24-Hour Ave Exceeded 14 pphm	1-1 Times:	.6 Percent

1-Hour Peak 61-63 3-Hour Peak 37-40 24-Hour Peak 9.5-11.6

Probe Bb GM = 1.4-1.9 SGD = 3.6, 2.3 Arithmetic Mean = 2.9-3.0

1-Hour Ave Exceeded 25 pphm	11-19 Times:	.3-.5 Percent
3-Hour Ave Exceeded 50 pphm	1-1 Times:	.1 Percent
24-Hour Ave Exceeded 10 pphm	1-1 Times:	.6 Percent
24-Hour Ave Exceeded 14 pphm	1-1 Times:	.6 Percent

1-Hour Peak 41-51 3-Hour Peak 25-33 24-Hour Peak 7.0-9.6

Probe Cc GM = 3.7-3.9 SGD = 3.1, 2.7 Arithmetic Mean = 7.3-7.3

1-Hour Ave Exceeded 25 pphm	131-166 Times:	3.8-4.8 Percent(a)
3-Hour Ave Exceeded 50 pphm	9-14 Times:	.8-1.2 Percent(b)
24-Hour Ave Exceeded 10 pphm	27-32 Times:	17.2-20.4 Percent(a)
24-Hour Ave Exceeded 14 pphm	8-14 Times:	5.1-8.9 Percent(b)

1-Hour Peak 127-162 3-Hour Peak 82-99 24-Hour Peak 27-30

Probe Dc GM = 6.5-6.7 SGD = 2.9, 2.5 Arithmetic Mean = 11.4-11.4

1-Hour Ave Exceeded 25 pphm	363-431 Times:	10.5-12.4 Percent(a)
3-Hour Ave Exceeded 50 pphm	43-54 Times:	3.7-4.6 Percent(b)
24-Hour Ave Exceeded 10 pphm	71-82 Times:	45.2-52.2 Percent(a)
24-Hour Ave Exceeded 14 pphm	51-53 Times:	32.5-33.8 Percent(b)

1 Hour Peak 264-353 3-Hour Peak 171-216 24-Hour Peak 60-74

Probe Db GM = 5.8-6.1 SGD = 3.2, 2.8 Arithmetic Mean = 11.7-11.7

1-Hour Ave Exceeded 25 pphm	335-394 Times:	9.7-11.4 Percent(a)
3-Hour Ave Exceeded 50 pphm	40-49 Times:	3.4-4.2 Percent(b)
24-Hour Ave Exceeded 10 pphm	65-74 Times:	41.4-47.1 Percent(a)
24-Hour Ave Exceeded 14 pphm	46-50 Times:	29.3-31.8 Percent(b)

1-Hour Peak 234-313 3-Hour Peak 157-222 24-Hour Peak 59-73

(a) violates Montana standards

(b) violates Federal standards

(c) possible violation of Montana standards

TABLE 9.2b. ZAPS II SEASONAL SUMMARY, 1976
(pphm SO_2)

Probe Ac GM = .2-1.4 SGD = 3.4, 1.2 Arithmetic Mean = .5-1.4

1-Hour Ave Exceeded 25 pphm	0 Times:	0 Percent
3-Hour Ave Exceeded 50 pphm	0 Times:	0 Percent
24-Hour Ave Exceeded 10 pphm	0 Times:	0 Percent
24-Hour Ave Exceeded 14 pphm	0 Times:	0 Percent

1-Hour Peak 6.0-6.0 3-Hour Peak 3.9-3.9 24-Hour Peak 1.7-1.7

Probe Bc GM = 2.6-2.9 SGD = 2.3, 1.8 Arithmetic Mean = 3.7-3.7

1-Hour Ave Exceeded 25 pphm	9-9 Times:	.24 Percent
3-Hour Ave Exceeded 50 pphm	0 Times:	0 Percent
24-Hour Ave Exceeded 10 pphm	2-3 Times:	1.2-1.7 Percent(a)
24-Hour Ave Exceeded 14 pphm	1-1 Times:	.6 Percent

1-Hour Peak 68-74 3-Hour Peak 40-43 24-Hour Peak 15-16

Probe Bb GM = 2.6-2.9 SGD = 2.5, 1.9 Arithmetic Mean = 4.0-4.0

1-Hour Ave Exceeded 25 pphm	8-9 Times:	.2-.2 Percent
3-Hour Ave Exceeded 50 pphm	0 Times:	0 Percent
24-Hour Ave Exceeded 10 pphm	2-3 Times:	1.2-1.7 Percent(a)
24-Hour Ave Exceeded 14 pphm	0 Times:	0 Percent

1-Hour Peak 60-62 3-Hour Peak 35-36 24-Hour Peak 13-14

Probe Cc GM = 4.1-4.4 SGD = 2.3, 1.9 Arithmetic Mean = 5.5-5.5

1-Hour Ave Exceeded 25 pphm	59-63 Times:	1.6-1.7 Percent(a)
3-Hour Ave Exceeded 50 pphm	2-2 Times:	.2 Percent
24-Hour Ave Exceeded 10 pphm	12-16 Times:	7.0-9.3 Percent(a)
24-Hour Ave Exceeded 14 pphm	5-5 Times:	2.9 Percent(b)

1-Hour Peak 110-125 3-Hour Peak 67-72 24-Hour Peak 24-27

Probe Cb GM = 4.0-4.3 SGD = 2.5, 2.0 Arithmetic Mean = 5.8-5.9

1-Hour Ave Exceeded 25 pphm	43-47 Times:	1.2-1.3 Percent(a)
3-Hour Ave Exceeded 50 pphm	1-1 Times:	.1 Percent
24-Hour Ave Exceeded 10 pphm	9-10 Times:	5.2-5.8 Percent(a)
24-Hour Ave Exceeded 14 pphm	1-1 Times:	.6 Percent

1-Hour Peak 87-87 3-Hour Peak 59-60 24-Hour Peak 19-19

Probe Dc GM = 6.4-6.9 SGD = 3.0, 2.3 Arithmetic Mean = 10.6-10.6

1-Hour Ave Exceeded 25 pphm	286-289 Times:	7.8-7.8 Percent(a)
3-Hour Ave Exceeded 50 pphm	11-14 Times:	.9-1.1 Percent(b)
24-Hour Ave Exceeded 10 pphm	72-72 Times:	41.9 Percent(a)
24-Hour Ave Exceeded 14 pphm	37-38 Times:	21.5-22.1 Percent(b)

1-Hour Peak 199-225 3-Hour Peak 117-130 24-Hour Peak 44-48

Probe Db GM = 7.0-7.5 SGD = 3.3, 2.7 Arithmetic Mean = 14.0-14.0

1-Hour Ave Exceeded 25 pphm	366-371 Times:	9.9-10.0 Percent(a)
3-Hour Ave Exceeded 50 pphm	12-14 Times:	1.0-1.1 Percent(b)
24-Hour Ave Exceeded 10 pphm	83-84 Times:	48.8-48.3 Percent(a)
24-Hour Ave Exceeded 14 pphm	46-47 Times:	26.7-27.3 Percent(b)

1-Hour Peak 193-218 3-Hour Peak 113-126 24-Hour Peak 43-47

(a) violates Montana standards

(b) violates Federal standards

TABLE 9.2c. ZAPS I SEASONAL SUMMARY, 1977
(pphm SO_2)

Probe Ac GM = .2-1.6 SGD = 4.5, 1.7 Arithmetic Mean = 1.1-2.1

1-Hour Ave Exceeded 25 pphm	2-5 Times:	0-.1 Percent
3-Hour Ave Exceeded 50 pphm	0 Times:	0 Percent
24-Hour Ave Exceeded 10 pphm	0 Times:	0 Percent
24-Hour Ave Exceeded 14 pphm	0 Times:	0 Percent

1-Hour Peak 26-28 3-Hour Peak 20-21 24-Hour Peak 3.6-4.6

Probe Bd GM = 3.5-3.7 SGD = 2.1, 2.4 Arithmetic Mean = 5.3-5.5

1-Hour Ave Exceeded 25 pphm	70-104 Times:	1.7-2.5 Percent(a)
3-Hour Ave Exceeded 50 pphm	0 Times:	0 Percent
24-Hour Ave Exceeded 10 pphm	9-15 Times:	5.0-8.8 Percent(a)
24-Hour Ave Exceeded 14 pphm	1-2 Times:	5.1 Percent(d)

1-Hour Peak 55-66 3-Hour Peak 40-48 24-Hour Peak 15-16

Probe Bc GM = 3.2-3.4 SGD = 2.2, 2.1 Arithmetic Mean = 4.6-4.8

1-Hour Ave Exceeded 25 pphm	68-85 Times:	1.6-2.0 Percent(a)
3-Hour Ave Exceeded 50 pphm	0 Times:	0 Percent
24-Hour Ave Exceeded 10 pphm	9-11 Times:	5.0-6.1 Percent(a)
24-Hour Ave Exceeded 14 pphm	3-5 Times:	1.6-2.8 Percent(b)

1-Hour Peak 51-56 3-Hour Peak 38-41 24-Hour Peak 19-21

Probe Bb GM = 3.7-3.9 SGD = 2.5, 2.4 Arithmetic Mean = 6.0-6.3

1-Hour Ave Exceeded 25 pphm	92-122 Times	2.2-2.9 Percent(a)
3-Hour Ave Exceeded 50 pphm	0 Times:	0 Percent
24-Hour Ave Exceeded 10 pphm	15-22 Times:	8.3-12.1 Percent(a)
24-Hour Ave Exceeded 14 pphm	3-4 Times:	1.7-2.2 Percent(b)

1-Hour Peak 57-66 3-Hour Peak 42-48 24-Hour Peak 18-19

Probe Cc GM = 5.3-5.6 SGD = 2.7 , 2.7 Arithmetic Mean = 9.5-10.2

1-Hour Ave Exceeded 25 pphm	289-333 Times:	6.9-7.9 Percent(a)
3-Hour Ave Exceeded 50 pphm	4-10 Times:	.3-.7 Percent(b)
24-Hour Ave Exceeded 10 pphm	57-62 Times:	31.5-34.3 Percent(a)
24-Hour Ave Exceeded 14 pphm	21-24 Times:	11.6-13.3 Percent(b)

1-Hour Peak 98-102 3-Hour Peak 64-71 24-Hour Peak 32-36

Probe Dc GM = 8.3-9.4 SGD = 3.1, 2.5 Arithmetic Mean = 14.1-15.3

1-Hour Ave Exceeded 25 pphm	606-677 Times:	14.4-16.1 Percent(a)
3-Hour Ave Exceeded 50 pphm	43-66 Times:	3.0-4.7 Percent(b)
24-Hour Ave Exceeded 10 pphm	120-126 Times:	66.3-69.6 Percent(a)
24-Hour Ave Exceeded 14 pphm	72-85 Times:	39.8-47.0 Percent(b)

1-Hour Peak 137-161 3-Hour Peak 96-116 24-Hour Peak 43-51

Probe Db GM = 7.0-7.5 SGD = 2.6, 2.5 Arithmetic Mean = 11.5-12.5

1-Hour Ave Exceeded 25 pphm	487-553 Times:	11.6-13.2 Percent(a)
3-Hour Ave Exceeded 50 pphm	40-50 Times:	2.8-3.5 Percent(b)
24-Hour Ave Exceeded 10 pphm	92-95 Times:	50.8-52.5 Percent(a)
24-Hour Ave Exceeded 14 pphm	48-57 Times:	26.5-31.5 Percent(b)

1-Hour Peak 120-147 3-Hour Peak 88-104 24-Hour Peak 51-61

(a) violates Montana standards

(b) violates Federal standards

(c) possible violation of Montana standards

(d) possible violation of Federal standards

TABLE 9.2d. ZAPS II SEASONAL SUMMARY, 1977
(pphm SO_2)

Probe Ac GM = .4-1.6 SGD = 4.2, 1.2 Arithmetic Mean = 1.0-1.6

1-Hour Ave Exceeded 25 pphm	2.0 Times:	.05 Percent
3-Hour Ave Exceeded 50 pphm	0 Times:	0 Percent
24-Hour Ave Exceeded 10 pphm	0 Times:	0 Percent
24-Hour Ave Exceeded 14 pphm	0 Times:	0 Percent

1-Hour Peak 33-38 3-Hour Peak 12-14 24-Hour Peak 3.0-3.4

Probe Bc GM = 2.9-3.0 SGD = 2.1, 1.9 Arithmetic Mean = 4.1-4.3

1-Hour Ave Exceeded 25 pphm	42-68 Times:	1.1-1.7 Percent(a)
3-Hour Ave Exceeded 50 pphm	0 Times:	0 Percent
24-Hour Ave Exceeded 10 pphm	4-10 Times:	2.2-5.5 Percent(a)
24-Hour Ave Exceeded 14 pphm	0-2 Times:	0.0-1.1 Percent

1-Hour Peak 66-83 3-Hour Peak 38-45 24-Hour Peak 13-15

Probe Bb GM = 3.0-3.2 SGD = 2.5, 2.2 Arithmetic Mean = 5.1-5.4

1-Hour Ave Exceeded 25 pphm	55-85 Times:	1.4-2.1 Percent(a)
3-Hour Ave Exceeded 50 pphm	0-2 Times:	0.0-0.2 Percent(d)
24-Hour Ave Exceeded 10 pphm	7-14 Times:	3.9-7.7 Percent(a)
24-Hour Ave Exceeded 14 pphm	3-3 Times:	1.7 Percent(b)

1-Hour Peak 86-112 3-Hour Peak 46-57 24-Hour Peak 16-20

Probe Cc GM = 4.7-4.9 SGD = 2.2, 2.1 Arithmetic Mean = 6.7-7.6

1-Hour Ave Exceeded 25 pphm	233-274 Times:	5.8-6.8 Percent(a)
3-Hour Ave Exceeded 50 pphm	5-8 Times:	.4-.6 Percent(b)
24-Hour Ave Exceeded 10 pphm	47-50 Times:	26.0-27.6 Percent(a)
24-Hour Ave Exceeded 14 pphm	14-20 Times:	7.7-11.0 Percent(b)

1-Hour Peak 123-160 3-Hour Peak 82-99 24-Hour Peak 28-32

Probe Cb GM = 4.6-4.8 SGD = 2.3, 2.3 Arithmetic Mean = 7.0-7.4

1-Hour Ave Exceeded 25 pphm	237-279 Times:	5.9-7.0 Percent(a)
3-Hour Ave Exceeded 50 pphm	6-7 Times:	.4-.5 Percent(b)
24-Hour Ave Exceeded 10 pphm	46-49 Times:	25.4-27.1 Percent(a)
24-Hour Ave Exceeded 14 pphm	16-19 Times:	8.8-10.5 Percent(b)

1-Hour Peak 118-152 3-Hour Peak 74-88 24-Hour Peak 25-29

Probe Dc GM = 7.5-8.2 SGD = 2.7, 2.4 Arithmetic Mean = 11.9-13.0

1-Hour Ave Exceeded 25 pphm	493-540 Times:	12.3-13.5 Percent(a)
3-Hour Ave Exceeded 50 pphm	35-55 Times:	2.6-4.0 Percent(b)
24-Hour Ave Exceeded 10 pphm	100-108 Times:	55.3-59.7 Percent(a)
24-Hour Ave Exceeded 14 pphm	62-67 Times:	34.2-37.0 Percent(b)

1-Hour Peak 191-253 3-Hour Peak 150-186 24-Hour Peak 50-61

Probe Db GM = 7.2-8.1 SGD = 3.3, 2.8 Arithmetic Mean = 15.0-16.9

1-Hour Ave Exceeded 25 pphm	479-548 Times:	12.0-13.7 Percent(a)
3-Hour Ave Exceeded 50 pphm	42-56 Times:	3.1-4.1 Percent(b)
24-Hour Ave Exceeded 10 pphm	93-102 Times:	51.4-56.4 Percent(a)
24-Hour Ave Exceeded 14 pphm	61-72 Times:	33.7-39.8 Percent(b)

1-Hour Peak 217-290 3-Hour Peak 186-235 24-Hour Peak 63-77

(a) violates Montana standards

(b) violates Federal standards

(c) possible violation of Montana standards

(d) possible violation of Federal standards

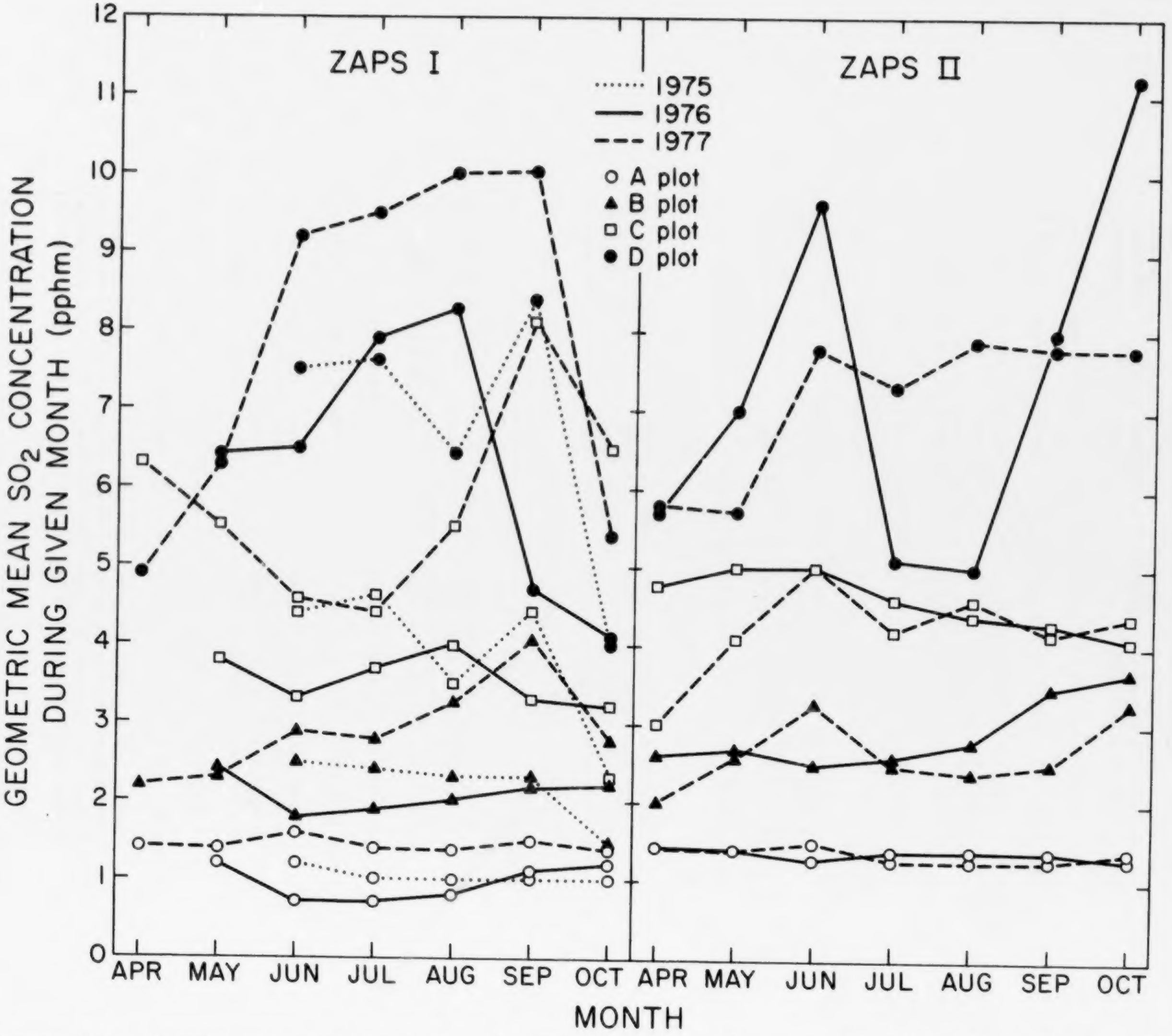


Figure 9.4. Intra-seasonal variation in SO$_2$ concentrations on the ZAPS plots (High run).

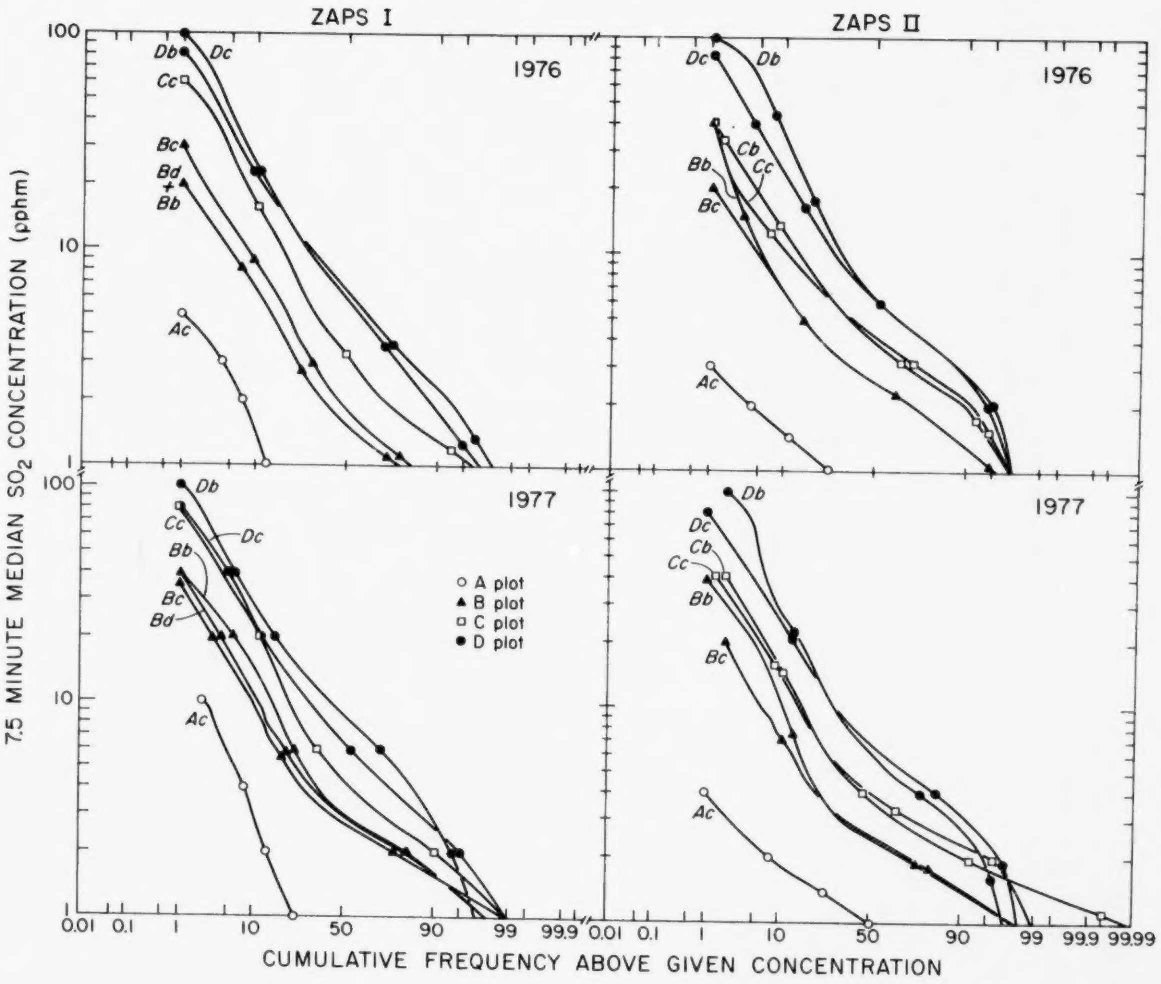


Figure 9.5. Cumulative frequency distributions of SO_2 concentrations at various locations on the ZAPS plots (Low run).

plot approximate each other. Separation of cumulative frequency distributions of different treatments plots is greatest near median concentrations and least at the extremely high and low concentrations. On ZAPS I, frequencies of high SO_2 concentrations in the C and D treatment plots converged during 1977. On ZAPS II, frequencies of high concentrations on plot B, monitoring location b converged with those of plot C during both 1976 and 1977.

Diel Patterns

The diel pattern at location c, averaged over each season, is shown in Figure 9.6. Each point represents the seasonal GM of all 7.5 minute averages occurring during a particular hour of the day. The unusually high concentrations observed on ZAPS I, C in 1977 were apparently caused by a monitoring problem during part of the season. Figure 9.7 shows the corresponding SGD's for ZAPS II, 1977. Nocturnal concentrations are typically higher and more variable than diurnal concentrations.

Effect of Micrometeorological Variables on Real Time SO_2 Concentrations

We have generally assumed that ZAPS SO_2 concentrations varied with micrometeorological conditions. We have examined this relation for ZAPS II for the period April 17 through June 25, 1977. The variables considered were hourly averages of:

C	SO_2 concentration	pphm
U	wind speed	kmph
SR	scale reading proportional to solar radiation	volts
R	relative humidity	%
I	temperature-stability class	unitless

Variable I was determined by the difference, ΔT, between the air and ground temperature as follows:

$\Delta T < -1°F$	, I = 1	"Unstable"
$-1° F \leq \Delta T \leq 1° F$	, I = 2	"Neutral"
$\Delta T > 1° F$	, I = 3	"Stable"

Except for R, these variables are generally considered important determinants of large scale pollutant dispersion (Turner, 1969). Wind speed is usually the most important variable, primarily due to direct dilution, but also to the generally increased rate of vertical (turbulant) mixing with higher wind speeds. I and SR are typically used to classify vertical mixing conditions with more stable conditions (poor mixing and higher concentrations) being associated with higher I values and lower SR values. R was included to test whether relative humidity could significantly affect SO_2 concentrations by affecting surface sorption of SO_2.

The results of linear regressions of these variables with concentration are given in Table 9.3a. As expected, inverse wind speed (1/U) was most important, explaining 38% of the variability of C. Solar radiation (SR) was next in importance, accounting for another 6.5% of the variability. Variable

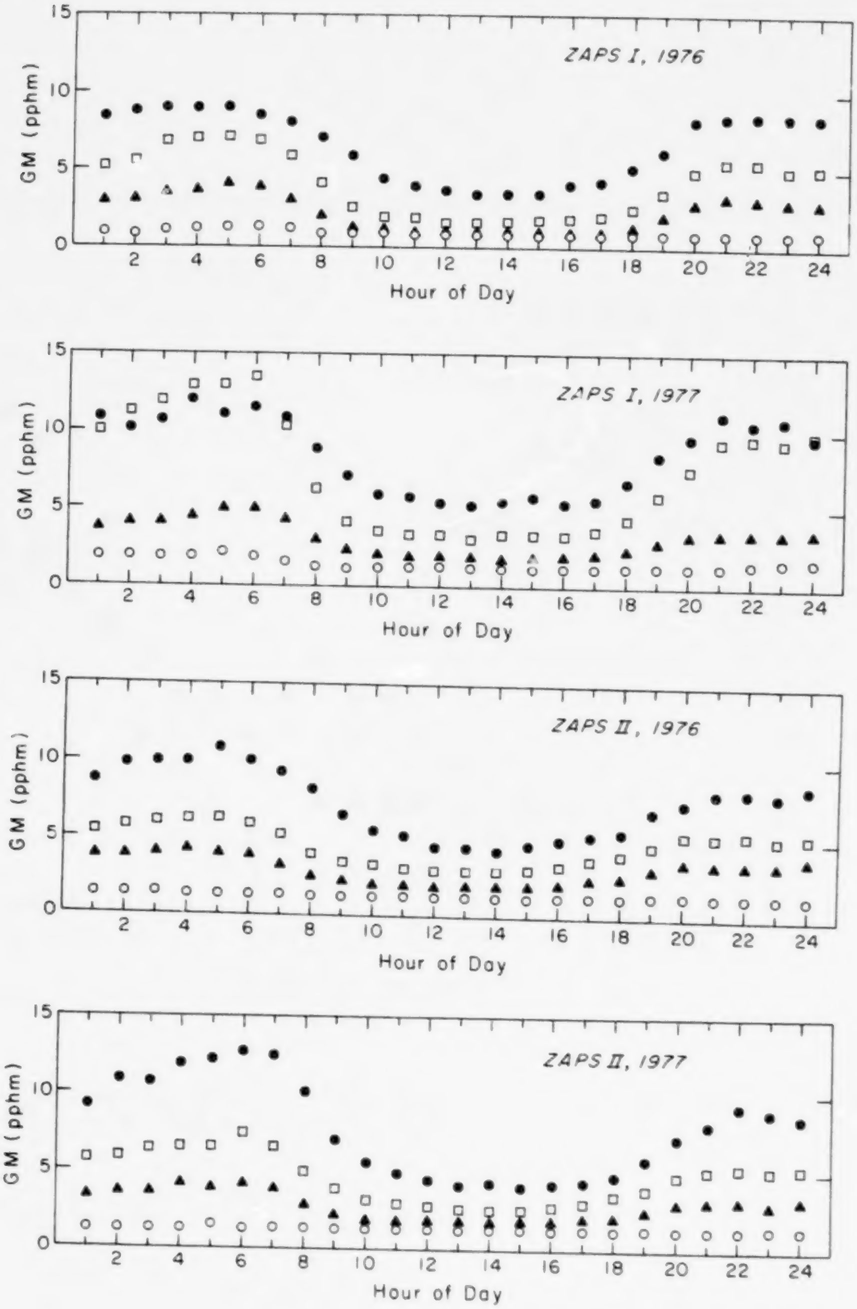


Figure 9.6. Geometric mean of 7.5 minute medians vs. hour of day. O = PLOT A, Δ = PLOT B, □ = PLOT C, ● = PLOT D (High run).

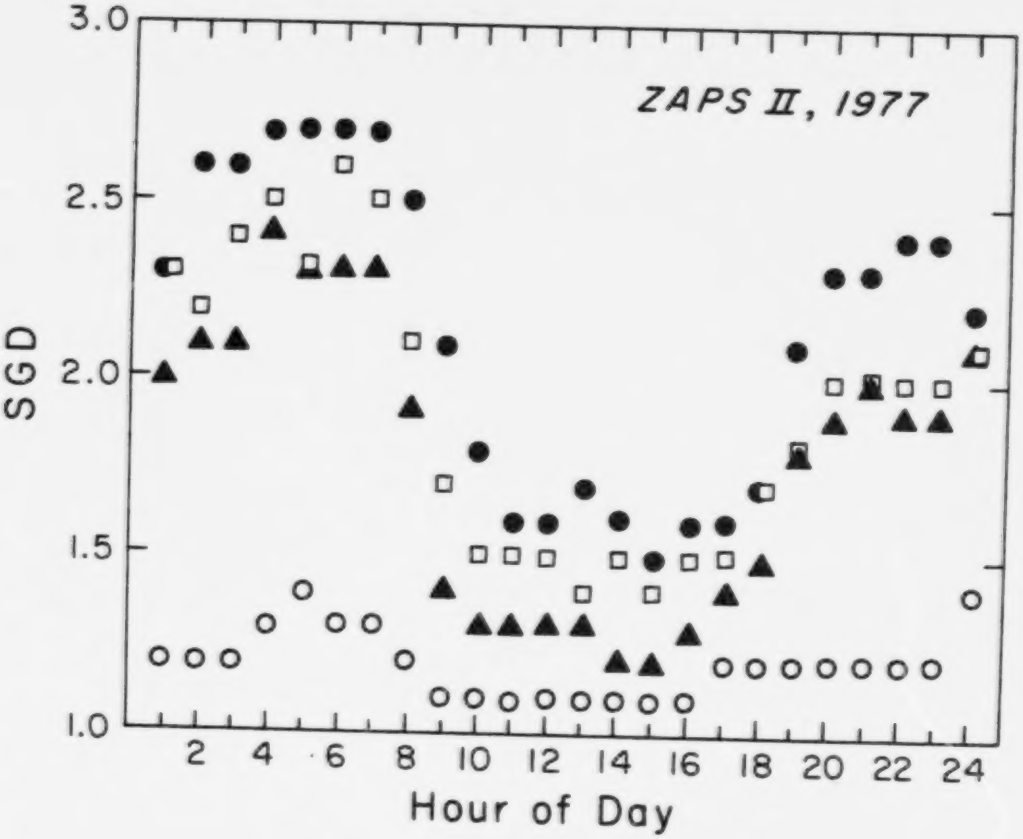


Figure 9.7. Standard geometric deviation of 7.5 minute medians for growing season vs. hour of day. O = PLOT A, Δ = PLOT B, □ = PLOT C, ● = PLOT D (High run).

TABLE 9.3. RELATION OF MICROMETEOROLOGICAL VARIABLES TO SO_2 CONCENTRATIONS

9.3a.

VARIABLES USED IN LINEAR REGRESSION OF VARIABLES

1/U	SR	I	R	r^2
X				.383
X		X		.418
X	X			.448
X	X	X		.456
X	X	X	X	.460

$$C = 4.08 - .017\ SR + 52.44/U$$

9.3b.

VARIABLES USED IN LINEAR REGRESSION OF LOG OF VARIABLES

U	1 + SR	I	r^2
X			.506
X		X	.536
X	X		.580
X	X	X	.604

$$\ln C = 3.76 - .0830 \ln (1 + SR) - .754 \ln U$$

9.3c.

RELATION BETWEEN ln U AND ln C FOR DAY AND NIGHT

$$C = a + b \ln U$$

	a	b
Night	4.2 ± .1	−0.95 ± .06
Day	3.1 ± .1	−0.62 ± .06

$$r^2 = .594$$

Confidence intervals are at 95% level.

I added 3.5% over 1/U, but less than 1% over 1/U and SR, and is of only minor importance. Although statistically significant, the addition of relative humidity R to the regression added very little (0.4%) to r^2.

Higher r^2 values were obtained by using the logarithms of C, U, and 1 + SR (Table 9.3b). The relative importance of the variables was the same, with ln U accounting for 51% of the variability of ln C, ln(1 + SR) another 7%, and I only 2% more than ln U and ln(1 + SR). One unexpected result is that the absolute value of the coefficient of ln U is less than 1; most models predict values greater than 1. The effect of wind speed is thus less than expected from straight dilution. This result reflects the persistance of low concentrations at low wind speeds during the daytime. For example, for U = 1.6 kmph, nocturnal concentrations are 75% higher than diurnal. The night-day difference in the dependance of concentration on windspeed is shown in Table 9.3c. At night, C is inversely proportional to U, as expected. During daylight hours, however, C decreases more slowly with increasing windspeed. One possibility is that mixing with clean air directly above ZAPS is greater during the day than at night. The regression equation given in 9.3c is a significantly better fit than that given in 9.3b, and is the equation of choice.

CONCLUSIONS

SO_2 concentrations on the ZAPS plots show substantial inter-season and intra-season variation. Concentrations are generally higher at night than during the day, but otherwise patterns of intra-seasonal variability are not consistent between ZAPS plots or between years. Intra-seasonal variation is greatest on D plots and least on A plots. Plots A, B and C on ZAPS II seem to have had less variable fumigation histories than the comparable plots on ZAPS I.

Seasonal frequency distributions of SO_2 concentrations are approximately log-normal and show differences in the fumigation histories of the plots. Though median concentrations on the A plots are small, these plots are subject to short-term acute fumigations due to drift from other plots.

SO_2 concentrations on the plots are strongly correlated with the inverse of wind speed. Solar radiation and temperature stability class, and relative humidity are also statistically significantly correlated with SO_2 concentration but are considerably less important in accounting for variability than inverse wind speed.

ACKNOWLEDGEMENTS

The operation of the ZAPS sites was supervised in the field by Tom Gullett during 1977. Tom also performed the quality assurance procedures on the SO_2 monitoring system. Ted Fletcher performed much of the day to day maintenance of the fumigation system.

REFERENCES

Larsen, R. I. 1971. A Mathematical Model for Relating Air Quality Measurements to Air Quality Standards. E.P.A. Report AP-89.

Lee, J. J. and R. A. Lewis. 1978. Zonal Air Pollution System, Design and Performance. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Third Interim Report, E. M. Preston and R. A. Lewis, eds. EPA-600/3-78-021, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 332-344.

Turner, D. B. 1969. Workbook of Atmospheric Dispersion Estimates. U.S. Dept HEW. 84 pp.

Weber, D., K. B. Olsen, and J. D. Ludwick. 1978. Field Experience with Ambient Level Flame Photometric Sulfur Detectors. Manuscript, U.S. Environmental Protection Agency, Corvallis, Oregon. 13 p.

SECTION 10

SPATIAL VARIATION OF SULFUR DIOXIDE CONCENTRATIONS ON ZAPS DURING THE 1977 FIELD SEASON

E. M. Preston and T. L. Gullett

ABSTRACT

Sulfur dioxide concentrations on the ZAPS plots were monitored with Huey sulfation plates at various points in horizontal and vertical space. These concentrations (as implied from sulfation rates) decreased with decreasing height above the ground within the plant canopy. Prevailing winds cause drift of SO_2 from the fumigated plots onto A-plots (Control). Though sulfation rates were highly variable at points within fumigated plots, only the D-plots (High treatment) showed statistically significantly different mean sulfation rates at different monitoring locations at 35 cm height. Differences on these plots correlate well with wind patterns. Stable dilution of SO_2 appears to be achieved within one to two meters of the gas delivery orifices.

INTRODUCTION

The distribution of introduced sulfur dioxide gas over the experimental plots has been a concern since the beginning of the ZAPS study. Until the present study, all monitoring of the sulfur fumigation has been accomplished with a Meloy 160-2 sulfur gas analyzer coupled with Teflon tubing from one to three locations on each of the fumigated plots. All sampling was done at 35 cm height above ground (approximate canopy height). In order to determine the horizontal and vertical distribution of SO_2, a series of Huey sulfation plates were set out at various locations and heights over the entire study site. The plates, which adsorb ambient sulfur dioxide and retain it as lead sulfate, were placed in the field for a specified length of time, collected at the end of the period and then analyzed for sulfate content. Results of this analysis were then used to describe the distribution pattern for the sulfur dioxide over the ZAPS site.

MATERIALS AND METHODS

The treatment plots, usually referred to as A, B, C, and D, represent control, low, medium and high concentration treatments respectively. In this study the intervals were also investigated as separate plots making a total of seven.

This study was composed of INTENSIVE and EXTENSIVE segments. The primary purpose of the EXTENSIVE study was to index the general relative concentrations of SO_2 at various points in vertical and horizontal space on the plots. Plates were widely spaced over each of the seven plots (Figure 10.1). One pole was placed in the center of each quadrant of each plot and a fifth pole placed in one of the two following positions: on plots A, B, C, and D, next to the real-time sulfur analyzer sample position c (Section 9) and on the interval plots, in the geometric center of the plot. On all poles, plates were placed at 15, 35, 75 and 150 centimeters above the ground. The exposure period in nearly all cases was 31 days. The study was repeated on both ZAPS I and II for 4 test periods (5-24-77 → 6-24-77; 6-25-77 → 7-25-77; 7-26-77 → 8-25-77; 8-26-77 → 9-25-77).

The major purpose of the INTENSIVE part of the study was to determine the dispersion pattern of the SO_2 immediately upon leaving the delivery system. Sulfation plates were placed in a tight pattern around five gas delivery orifices on plot B. The study area for the INTENSIVE study is shown in Figure 10.2. The spatial relationship between pole and orifice is shown in Figure 10.3. Poles had plates at 15, 35, 75 and 150 cm. The study was repeated for both ZAPS I and II for 3 test periods (7-05-77 → 8-05-77; 8-06-77 → 9-05-77; 9-05-77 → 10-06-77).

In all instances, the sulfation plates were placed into holders (adjustable Broom Clamp #2B, A.I. Platt Co., Fairfield, Conn.) attached to poles that had been driven into the ground. The plates were held parallel to the ground with the open face down. Sulfate determination of the exposed plates was conducted by Environmental Management Planning Laboratory in Nashville, Tenn.

RESULTS AND DISCUSSION

Extensive Study

Vertical Distribution of SO_2

The only useable data for vertical distribution of SO_2 plates at 15 cm above ground was obtained during the fourth test period. During other test periods, low level plates were destroyed by insects and rodents. Therefore only values from test period 4 were used to compare gas concentrations at different heights. The average sulfation rates for each plot at each height are shown in Figure 10.4. On ZAPS I, the highest gas level occurred at 75 cm followed closely by the 35 cm height. The gas escape orifices on the ZAPS I site are located on the sides of the delivery pipes and direct the gas parallel

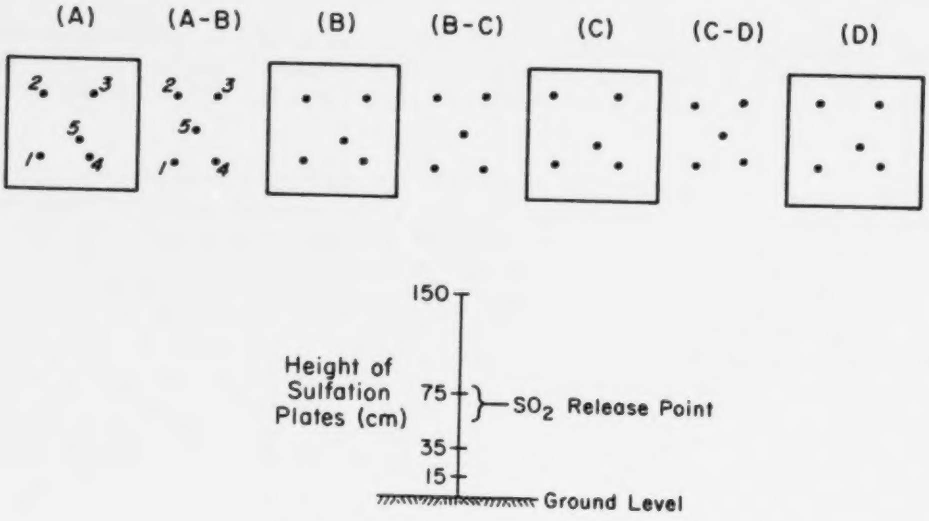

(A)
(A-B)
(B)
(B-C)
(C)
(C-D)
(D)
2
3
5
1
4
Height of Sulfation Plates (cm)
150
75
35
15
SO2 Release Point
Ground Level

Figure 10.1. Location of sulfation plate monitoring positions on the EXTENSIVE grid.

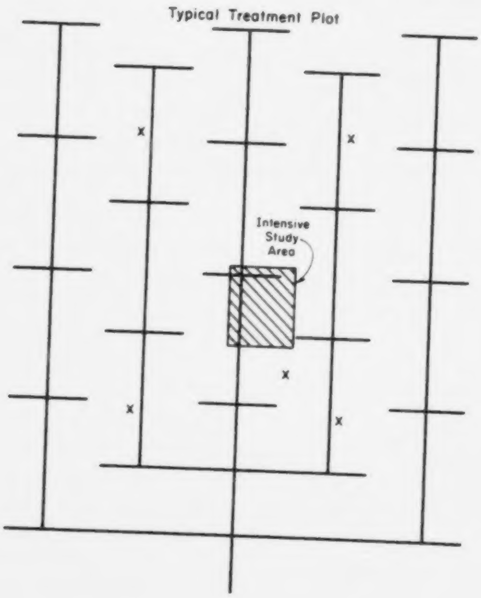

Typical Treatment Plot
Intensive Study Area
x
x
x
x
x

Figure 10.2. Location of INTENSIVE study area on ZAPS, plot B. Monitoring locations for the EXTENSIVE study on this plot are also indicated.

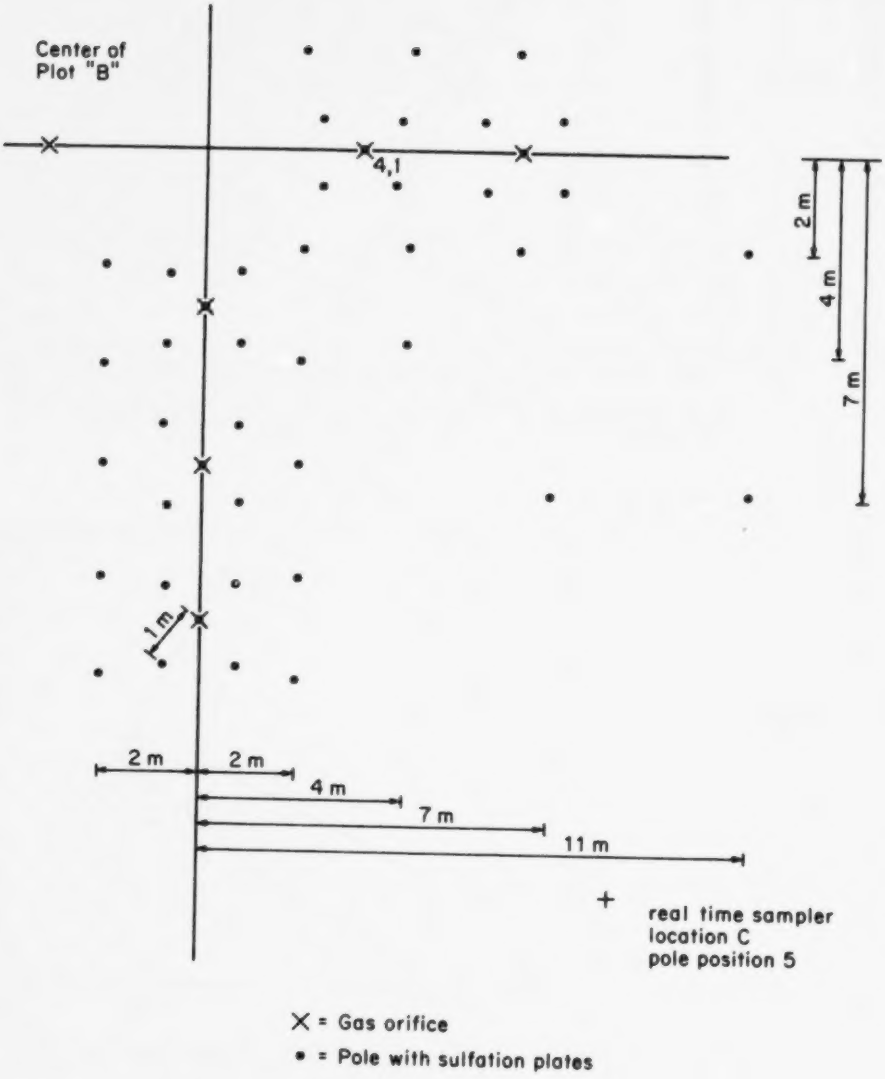

Center of
Plot "B"
4,1
2 m
4 m
7 m
2 m
2 m
4 m
7 m
11 m
1 m
real time sampler
location C
pole position 5
X = Gas orifice
• = Pole with sulfation plates

Figure 10.3. Spatial relationship of gas delivery orifices and sulfation plate monitoring locations in the INTENSIVE study.

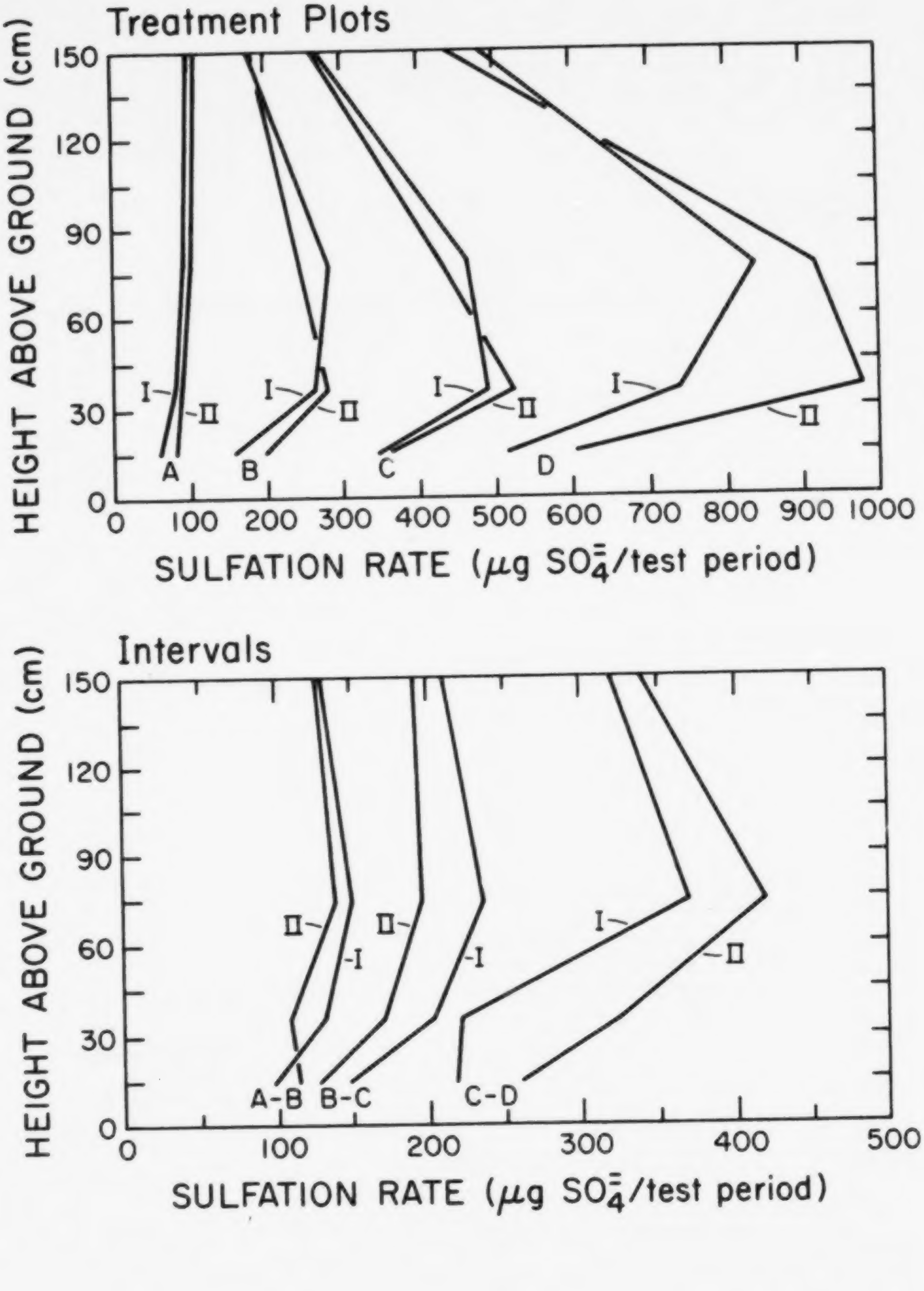


Figure 10.4. Vertical profile of sulfation rates during test period 4 (8-26-77 → 9-25-77).

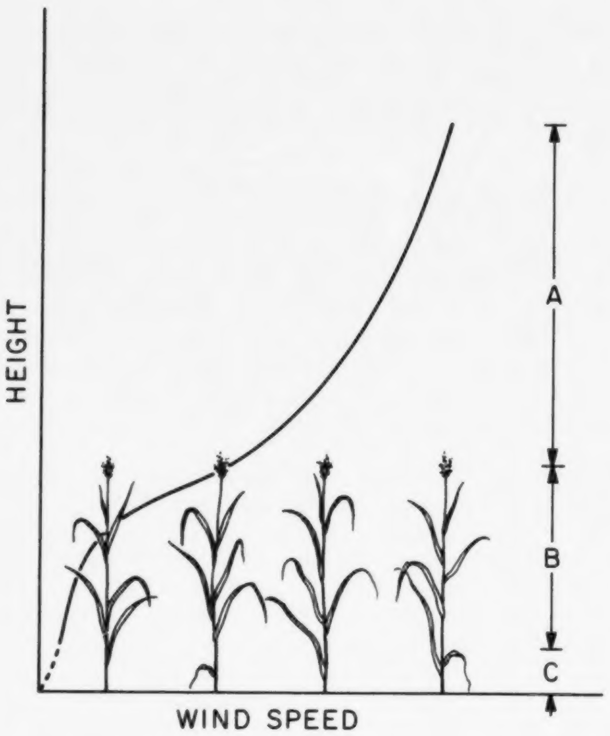


Figure 10.5. Hypothetical vertical profile of wind speeds in a grassland canopy: (A) logarithmic profile, (B) exponential profile, (C) logarithmic profile.

to the ground while the ZAPS II gas is directed toward the ground by orifices located in the bottom of the delivery pipes which are also set slightly closer to the ground. These differences in construction may account for the different stratification patterns seen at the 35-75 cm level between the two sites. On both ZAPS I and II, the 15 cm level received about 75% and the 150 cm level received about 85% as much gas as did the 35 cm level.

Several factors contribute to the vertical distribution of sulfation rates shown in Figure 10.4. Gases are transported by a combination of diffusion and air movement. Canopy air flows can largely determine the rate of SO_2 transport to biota from the ZAPS SO_2 delivery system. Figure 10.5 shows a hypothetical vertical distribution of wind speeds above and within a grassland canopy. Horizontal winds flowing over the canopy surface are slowed by frictional drag on the vegetation. Immediately above the canopy, the mean horizontal wind velocity decreases logarithmically with decreasing height. Within the canopy, a canopy-eddy layer exists in which wind speed decreases exponentially. In the lowest part of the plant-air layer a logarithmic wind profile resumes with wind speed decreasing to zero at ground level (Inoue, 1963). The degree of vertical mixing of SO_2 at various vertical distances from the SO_2 delivery pipes on the ZAPS plots should vary directly with wind speeds at those heights and therefore should decrease with decreasing height above ground level.

The sulfation rate of sulfation plates appears to vary directly with the square root of wind speed (Liang *et al.*, 1973). Thus, if wind speed decreases with height in the canopy, measured sulfation rate would be expected to decrease moderately with height even if SO_2 concentrations remained constant. SO_2 concentrations would also be expected to decrease with decreasing height because transport of the plume by turbulent mixing decreases with decreasing height and some SO_2 is removed from the air by adsorption and/or absorption by the biota within the canopy. The observed vertical profile of sulfation rates (Figure 10.4) are consistent with these expectations.

It is of interest to determine whether or not the vertical profile of sulfation rates within the canopy on the fumigated plots is substantially different from that which might be expected during fumigation from a power plant plume. It is possible that the observed vertical profile of sulfation on the fumigated plots has been unduly influenced by turbulent mixing generated by the delivery of SO_2 under pressure from the gas delivery system. Some insight can be gained by comparing the vertical distribution of sulfation rates on the fumigated plots with those on the intervals, which are not influenced by gas delivery systems. Mixing above the canopy is potentially influenced by ambient turbulent air flow, and on the fumigated plots, by turbulence caused by the pressurized delivery of SO_2. Below the canopy, turbulent air flow is influenced by these two factors and also modified by the canopy. If the turbulence caused by the gas delivery system were a major influence on sulfation rates, the vertical profiles in sulfation rates on fumigated plots should be different from those on intervals. Figure 10.6 shows the change in sulfation rates between 35 cm and 15 cm within the canopy as a function of sulfation rate at 35 cm. This change is directly linearly proportional to the sulfation rates at 35 cm height. The proportionality on the intervals is similar to that on the fumigated plots. This suggests that the canopy is the dominant moderating influence and that the gas delivery system influences the vertical distribution of sulfation rates very little. This is not true, however, for sulfation rates measured above the canopy (Figure 10.6). While the change in sulfation rates between 35 and 150 cm height are directly proportional to the sulfation rate at 35 cm on the fumigated plots, the relationship does not hold for the intervals. The SO_2 delivery systems apparently have a major influence on the vertical profile of sulfation rates measured above the canopy. The data available suggest that the vertical profile of SO_2 concentrations implied by the vertical profile of sulfation rates within the canopy is primarily the result of micrometeorological and biological processes and is not an artifact of the design of the gas delivery systems.

If SO_2 exposure concentrations are vertically stratified within an ecosystem, quantification of the dose delivered to particular ecosystem components becomes a complex problem, particularly when these components are

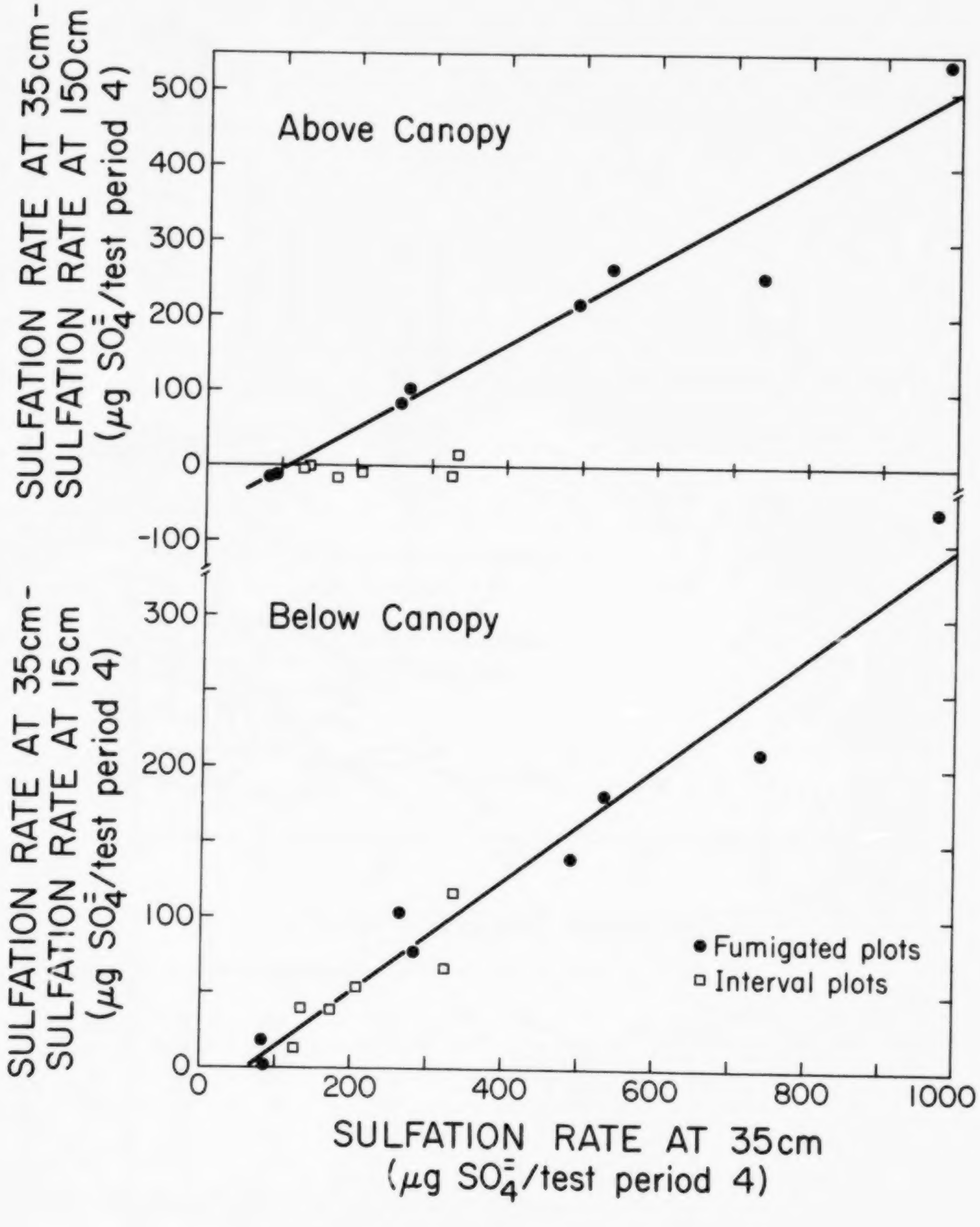


Figure 10.6. Vertical changes in sulfation rates (mean of 5 pole positions, test period 4) above and below the canopy as a function of sulfation rate at canopy height.

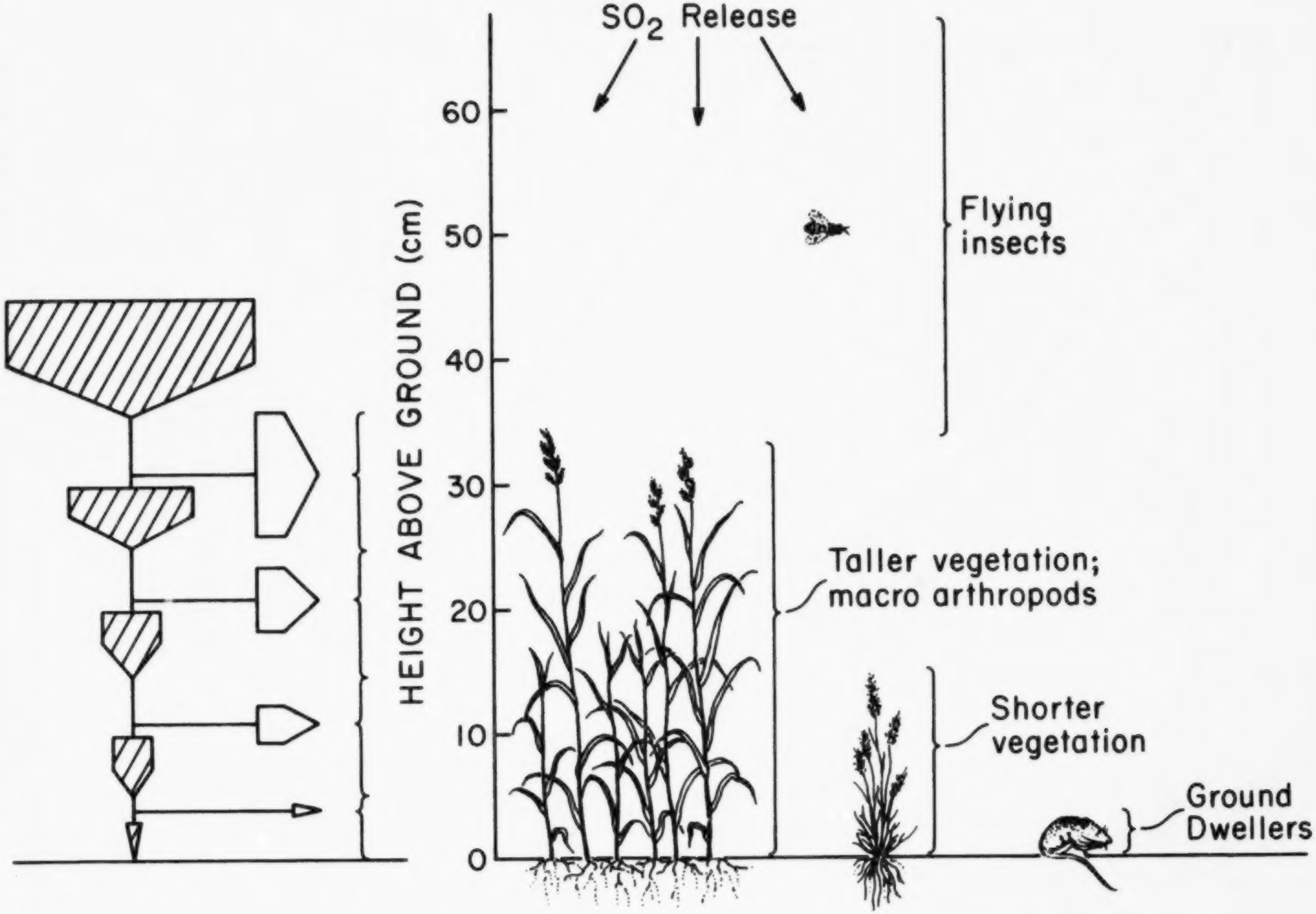

SO2 Release
HEIGHT ABOVE GROUND (cm)
60
50
40
30
20
10
0
Flying insects
Taller vegetation; macro arthropods
Shorter vegetation
Ground Dwellers

Figure 10.7. Hypothetical SO_2 flux in native grassland canopy. Solid arrows represent relative amounts of SO_2 reaching various strata. Open arrows represent relative amounts of SO_2 absorbed at biotic receptor sites within various strata.

also vertically stratified spatially and/or temporally. In a native grassland ecosystem there is considerable vertical stratification of ecosystem components. Figure 10.7 illustrates a hypothetical gas exchange system within a native grassland canopy. For discussion, the canopy has been divided into 5 strata. The width of arrows in the left-hand half of the diagram represents the magnitude of SO_2 fluxes through the strata. The decreasing flux with height (solid arrows) results from reduced vertical mixing and the net vertical removal of SO_2 by tissues within each stratum (open arrows). The relative magnitudes of vertical fluxes versus tissue fluxes have been assigned arbitrarily. These would be expected to vary temporally and probably spatially. The vertical flux gradient would vary with the degree of ventilation and tissue flux. Tissue flux would vary between strata depending upon the number of available SO_2 removal sites within each stratum.

The right-hand portion of Figure 10.7 indicates the vertical distribution of some of the important biotic components of a native grassland ecosystem on the ZAPS sites. Low flying insects would generally receive the greatest dose. Vegetation would receive a variety of dosages throughout its height. Dosages received by macroarthropods would vary temporally depending on their temporal patterns in the use of vertical space. Ground dwelling organisms would generally receive the lowest overall dose.

Horizontal SO_2 Distribution

To characterize the distribution of SO_2 concentrations in horizontal space on the ZAPS plots, we compared sulfation at the five pole positions at 35 cm height (roughly canopy height) with oneway ANOVA. Plot locations were considered treatments and test periods were considered replications.

On treatment plots, there were no statistically significant differences in sulfation rates among pole positions except on the D plots (Table 10.1, Figure 10.8). On the D plots, pole 1 had higher sulfation rates than other poles on ZAPS I and poles 1 and 4 had higher sulfation rates than others on ZAPS II. On ZAPS I, there is a trend for sulfation in pole positions 1 and 4 to be greater than that on poles 2 and 3. To see if this trend could be detected statistically, paired-t tests were used to compare summed average sulfation values (Table 10.1) for poles 1 and 4 with the summed values for poles 2 and 3. Test periods were considered replications. Once again, only the D plots showed a significant difference (Table 10.2).

Eversman (Section 17) reports that sulfation (at 50 cm height) was greatest near the center of plot D on ZAPS I (Figure 10.8). The differences between Eversman's relative sulfation rates at pole positions and those reported here illustrate that locations with small differences in vertical position may have dramatic differences in relative SO_2 concentrations.

The two factors likely to have the greatest influence on the spatial distribution of SO_2 concentrations are the gas delivery system and wind. The gas delivery system was intended to deliver a spatially uniform concentration of SO_2 at canopy height. However, any drop in pressure along the delivery pipes would cause locally lower concentrations of SO_2 to be delivered.

TABLE 10.1. MEAN SULFATION RATES AT VARIOUS POINTS IN HORIZONTAL SPACE ON THE ZAPS PLOTS

ZAPS I

Mean sulfation rates of four test periods
at 35 cm height at Pole positions

Plot	1	2	3	4	5	LSD (0.05)	F	P
				(μg $SO_4^=$/test period)				
A	50	40	69	38	41	58	0.44	NS
Interval A-B	74	67	90	119	76	75	0.69	NS
B	187	173	172	184	204	103	0.14	NS
Interval B-C	149	108	138	201	146	74	1.87	NS
C	386	370	338	378	364	160	0.11	NS
Interval C-D	236	169	209	392	242	112	5.20	<.01
D	1013	633	512	652	625	271	4.50	<.05
ZAPS II								
A	56	49	45	59	70	58	0.24	NS
Interval A-B	93	88	116	188	123	132	0.83	NS
B	228	228	204	174	193	108	0.42	NS
Interval B-C	118	108	154	170	146	51	2.31	NS
C	528	482	426	446	422	114	1.39	NS
Interval C-D	215	253	262	360	266	160	1.01	NS
D	1216	799	950	1271	597	230	13.72	<.01

TABLE 10.2. MEAN DIFFERENCES BETWEEN SUMMED SULFATION RATES FOR POLES 1 AND 4 AND SUMMED SULFATION RATES FOR POLES 2 AND 3

ZAPS I

Plots	A	B	C	D
$\bar{D}$* (μg $SO_4^=$)	20	25	-20	520
S_D (μg $SO_4^=$)	20	68	146	161
t	-2.03	0.75	-0.27	6.48
P	NS	NS	NS	<.01
		ZAPS II		
$\bar{D}$	21	-30	67	759
S_D	39	106	163	151
t	1.11	-.57	0.82	10.07
P	NS	NS	NS	<.005

*$\bar{D}$ = average difference in sulfation rates at 35 cm height between upper plot positions and lower plot positions !(pole positions 1 + 4) - (pole positions 2 + 3)1.

S_D = Standard deviation of $\bar{D}$.

t = paired t statistic.

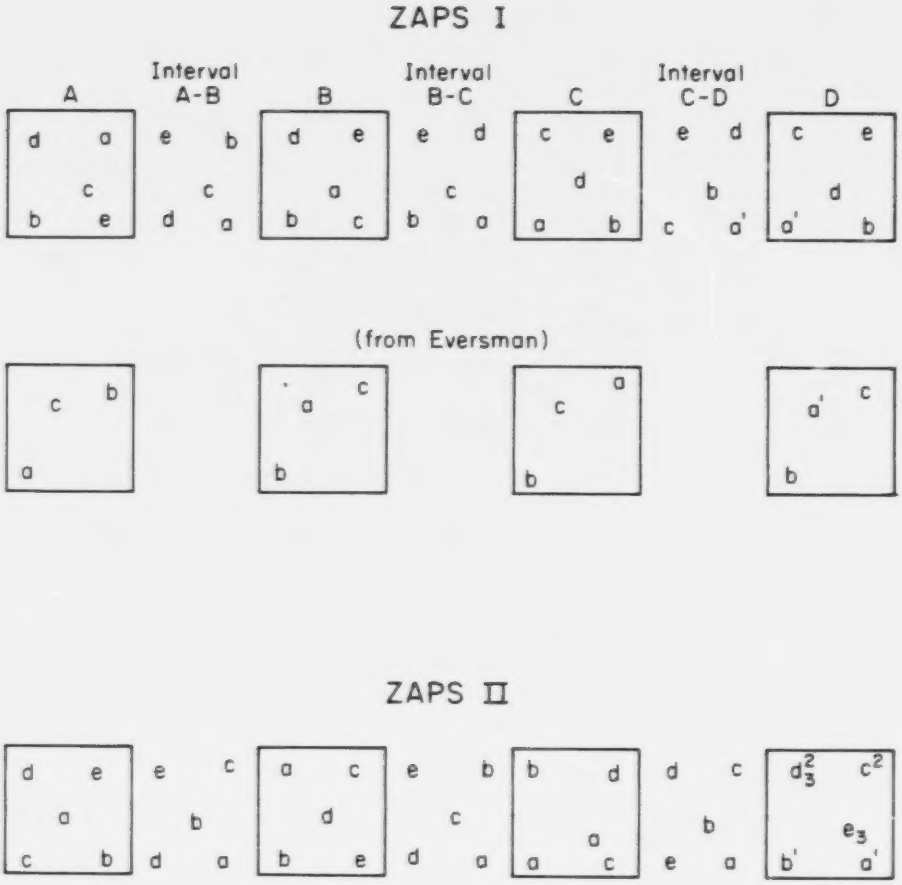

ZAPS I
A
Interval A-B
B
Interval B-C
C
Interval C-D
D
d a e b d e e d c e e d c e
c c a c d b d
b e d a b c b a a b c a' a' b
(from Eversman)
c b c a
a c
a b b b
a' c
ZAPS II
d e e c a c e b b d d c d_3^2 c^2
a b d c a b e_3
c b d a b e d a a c e a b' a'

Figure 10.8. Ranks of sulfation values at different positions on the ZAPS plots. Letters sharing the same super and/or subscripts are not significantly different from other letters within the same plot (P <.05). Ranks are based on average values for all test periods at 35 cm height. a → e = greatest sulfation rate → least sulfation rate.

Frictional forces would increase the chances of significant pressure drop with increasing distance from the pump. Such a pressure drop could cause the observed trend on the ZAPS I and the D plots (Figure 10.8).

Similar results could also be produced by wind patterns. If wind displacement of SO_2 were equal (on the average) in all directions, positions at the periphery of fumigated plots would tend to get diluted while interior positions receiving drift from the periphery, would tend to have higher SO_2 concentrations. If wind displacement were not equal in all directions, peripheral points receiving the greatest dilution would have the lowest SO_2 concentrations while those points receiving the greatest drift should have the highest average concentrations. In addition, all plots may have their SO_2 supplemented by SO_2 drifting from other fumigated plots. The magnitude of this can be gauged somewhat by sulfation measured on the A plots since these receive SO_2 by drift alone. The seasonal average concentrations on the A plots were about 27% of those on adjacent B plots (ZAPS I = 25.9%, ZAPS II = 27.2%).

Wind affects the measured sulfation values in at least two ways. First, in the ideal case where a single gaseous material at constant ambient concentration and temperature is being sampled, the theoretical model proposed by Liang et al. (1973) suggests that sulfation rate varies directly with the square root of wind speed. Secondly, wind dilutes and mixes SO_2 delivered to the plots. Under theoretically ideal conditions, one might expect SO_2 concentration to be reduced in proportion to the reciprocal of some power of wind speed equal or greater than one. Based on the above two assumptions, we developed an index of sulfation potential which varies with wind speed and its duration. One sulfation hour was defined as the square root of wind speed for that hour divided by wind speed for that hour ($\frac{\sqrt{ws}}{ws} = \frac{1}{\sqrt{ws}}$). With this index, a light wind produces a low sulfation potential but also a weak SO_2 dilution. A stronger wind will produce a high SO_2 absorbance potential but a much stronger SO_2 dilution yielding a lower potential sulfation rate. The dilution factor quickly over powers the increased SO_2 absorption potential caused by increased wind speed. This effect would be even more dramatic if it were assumed that dilution varied with the reciprocal of a power of wind speed greater than one. The higher the index, the higher the expected sulfation rate during that hour.

To characterize how wind might be expected to influence measured sulfation at various locations, the Sulfation Potential Index for each wind direction in each test period was calculated (Table 10.3). Wind speed and direction at two meters height measured in the B-C plot intervals was assumed typical of wind patterns on all plots. This assumption may be violated rather seriously, particularly as one approaches the canopy. Unfortunately, no more appropriate documentation of wind patterns on the plots exists. Wind speeds were divided into ten categories between 0.0 and 8.9 m/sec. The number of hours that wind speed in each category occurred from each of eight directions (N, NE, E, SE, S, SW, W, NW) in each test period was determined. The Sulfation Potential Index for each test period was calculated as the summation of the number of hours that winds occurred in each speed category multiplied by the reciprocal of the square root of average wind speed for that category for each compass

TABLE 10.3. SULFATION POTENTIAL INDICES* OF WINDS COMING FROM COMPASS DIRECTIONS DURING DIFFERENT TEST PERIODS

ZAPS I

(Values adjusted to a common total of 744 hours/test period)

$(\text{Hrs} \cdot (\text{m/sec})^{-\frac{1}{2}})$

Test Period	N	NE	E	SE	S	SW	W	NW	Total
				Wind Direction					
1	31	18	22	44	24	14	17	17	187
2	24	21	17	23	27	22	25	12	171
3	24	20	22	36	29	14	21	22	188
4	22	11	25	42	28	8	14	23	173
Total	101	70	86	145	108	58	77	74	

ZAPS II

(Values adjusted to a common total of 760 hrs/test period)

$(\text{Hrs} \cdot (\text{m/sec})^{-\frac{1}{2}})$

Test Period	N	NE	E	SE	S	SW	W	NW	Total
				Wind Direction					
1	20	22	46	14	16	15	41	16	190
2	18	25	34	21	12	9	48	14	181
3	21	28	30	15	11	14	52	20	191
4	29	17	47	15	12	10	35	22	187
Total	88	92	157	65	51	48	176	72	

$$\text{*Sulfation Potential Index} = \sum_{i=1}^{10} \frac{(\sqrt{ws_i})(Hrs_i)}{ws_i} = \sum_{i=1}^{10} \frac{Hrs_i}{\sqrt{ws_i}}$$

ws_i = average wind speed (m/sec) for the i^{th} category.

Hrs_i = the number of hrs that ws_i persisted during the given test period.

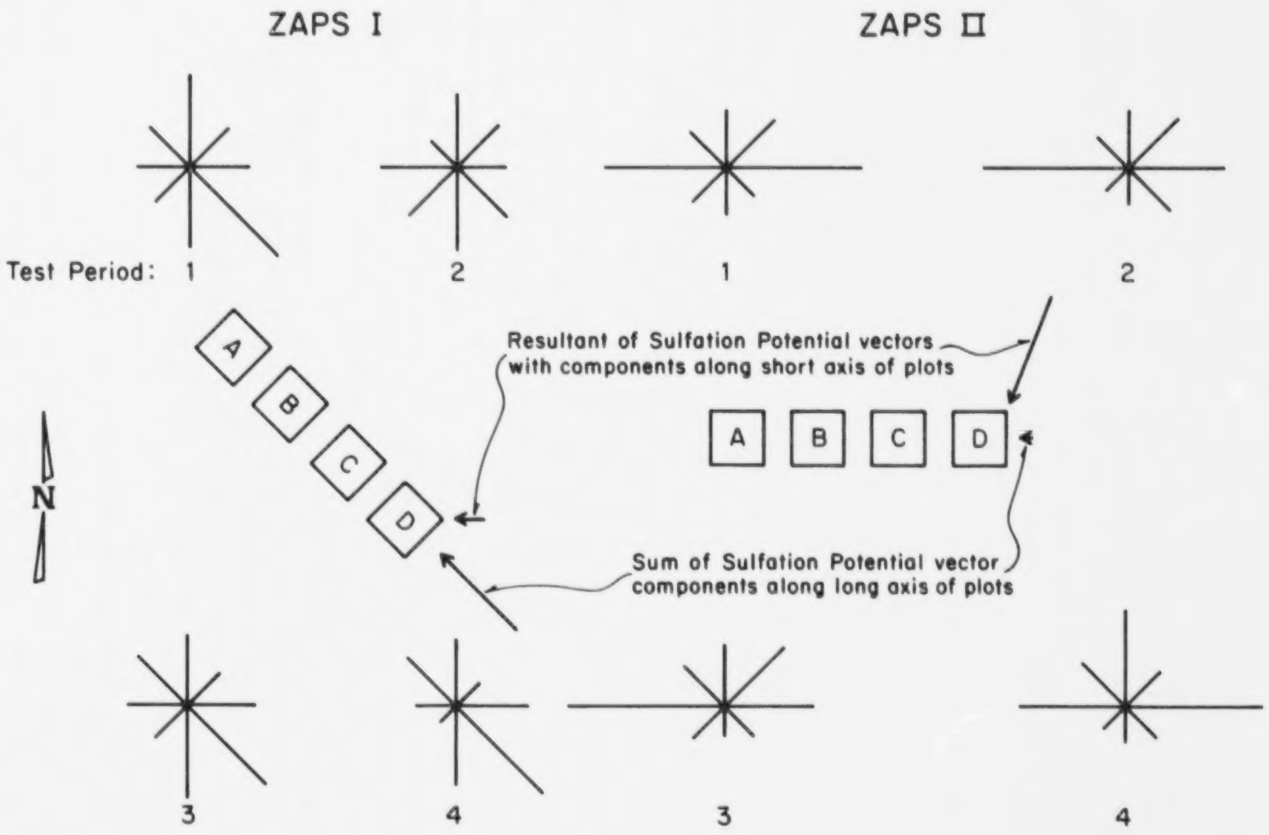

ZAPS I
ZAPS II
Test Period: 1
2
1
2
A
B
C
D
A
B
C
D
N
Resultant of Sulfation Potential vectors with components along short axis of plots
Sum of Sulfation Potential vector components along long axis of plots
3
4
3
4

Figure 10.9. Sulfation potential of winds coming from various directions during different test periods.

direction. Since sulfation rate should be proportional to this index, these index values indicate the potential effect on sulfation rates of winds from each wind direction during each test period.

Figure 10.9 illustrates the relative influence of winds from different directions on Sulfation Potential for the four test periods. The approximate orientation of the ZAPS plots is also indicated. Two composite vectors derived from the Sulfation Potential vectors are illustrated. In one, all vector components capable of contributing to SO_2 drift along the long axis of the ZAPS plots have been summed. This should indicate the net direction and sulfation potential to be expected. On both sites, the trend is for SO_2 to drift from higher concentration plots to lower concentration plots. On ZAPS I the tendency is large while on ZAPS II the tendency is slight.

The second vector indicated is the resultant of all vectors having components which could cause drift along the short axis of the plots. On ZAPS I this resultant would carry SO_2 diagonally across the plots and could cause the observed sulfation "hot spot" at pole 1, plot D (Table 10.1). On ZAPS II, drift with high sulfation potential is directed on less of a diagonal and could produce the observed more nearly equal sulfation rates at poles 1 and 4. Since the magnitude of this resultant is greater on ZAPS II than on ZAPS I, one would expect mean differences between summed sulfation rates for poles 1 and 4 and sumed sulfation rates for poles 2 and 3 to be greater on ZAPS II than on ZAPS I. This has been demonstrated (Table 10.2). Though the observed trends in sulfation seem to be explainable by wind patterns, deficiencies in the gas delivery system cannot be ruled out.

Figure 10.10 shows average sulfation for each test period on each plot at 35 cm height. An expected value is provided for comparison. On ZAPS II, plot D, this value is the mean sulfation rate for the four test periods. Expected values for the other plots on ZAPS II were computed assuming interplot ratios of 0:2:5:10 for A:B:C:D. On ZAPS I, expected values were computed similarly but only including data for test periods 1, 2, and 4 in computation of the expected value for plot D. Data for test period 3 on ZAPS I were unusually and unexplainably low. Therefore, separate expected values were computed for test period 3 applying the above procedure.

In general, SO_2 concentration ratios between fumigated plots are reasonably close to the hypothetical 0:2:5:10 delivery rates. Lower concentration plots tend to be higher than expected due probably to the net drift from the higher plots towards the lower plots indicated in Figure 10.9. Drift of SO_2 reaches the control plots and ambient concentrations average about one fourth of those of the B plots.

Conversion Factors For Sulfation Values

Pole position 5 for sulfation measurements coincides with monitoring location c for the real-time flame photometric analyzers on all experimental plots. Having both real-time SO_2 analyzers and sulfation plates monitoring SO_2 levels allowed computation of conversion factors for estimating mean SO_2 levels from sulfation data collected at other points on the plots. The

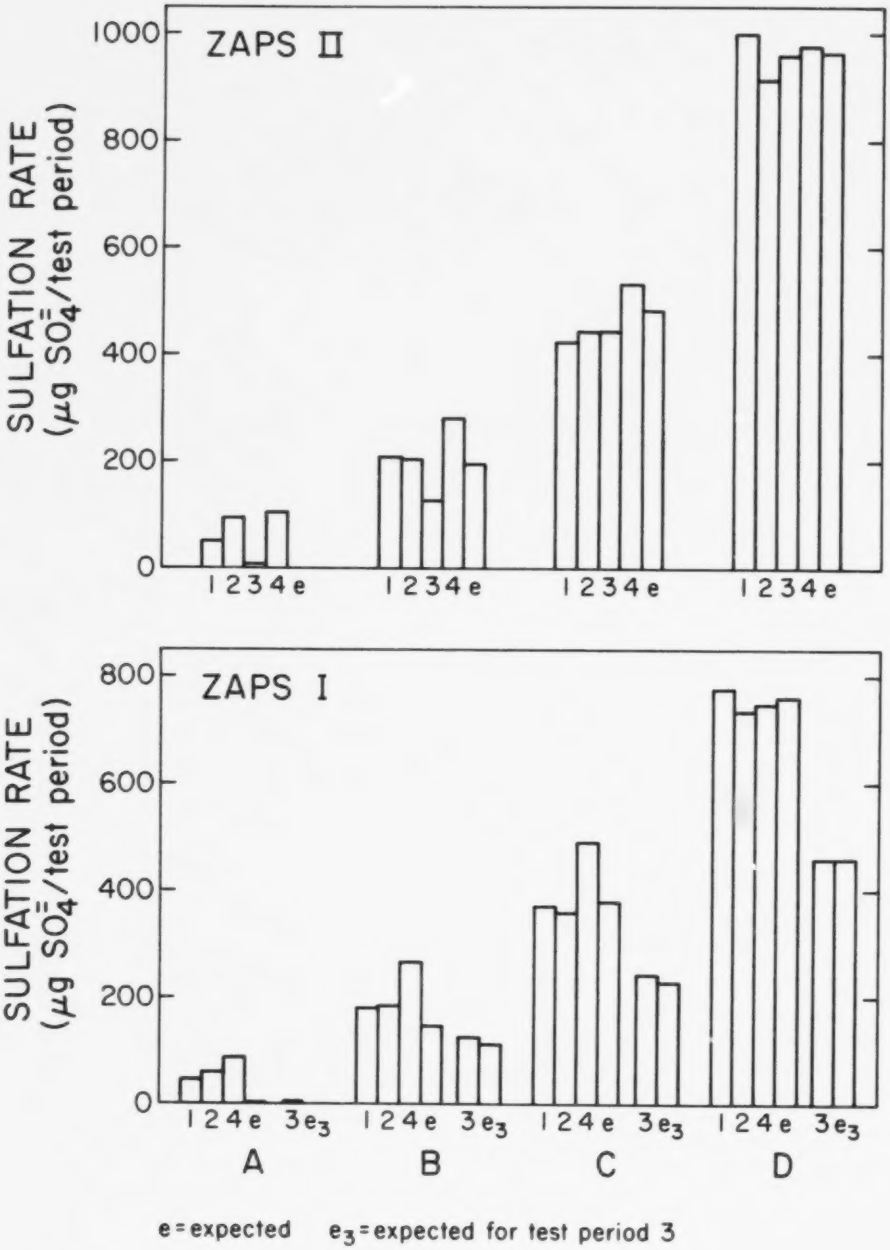


Figure 10.10. Average sulfation rates at 35 cm height on fumigated plots.

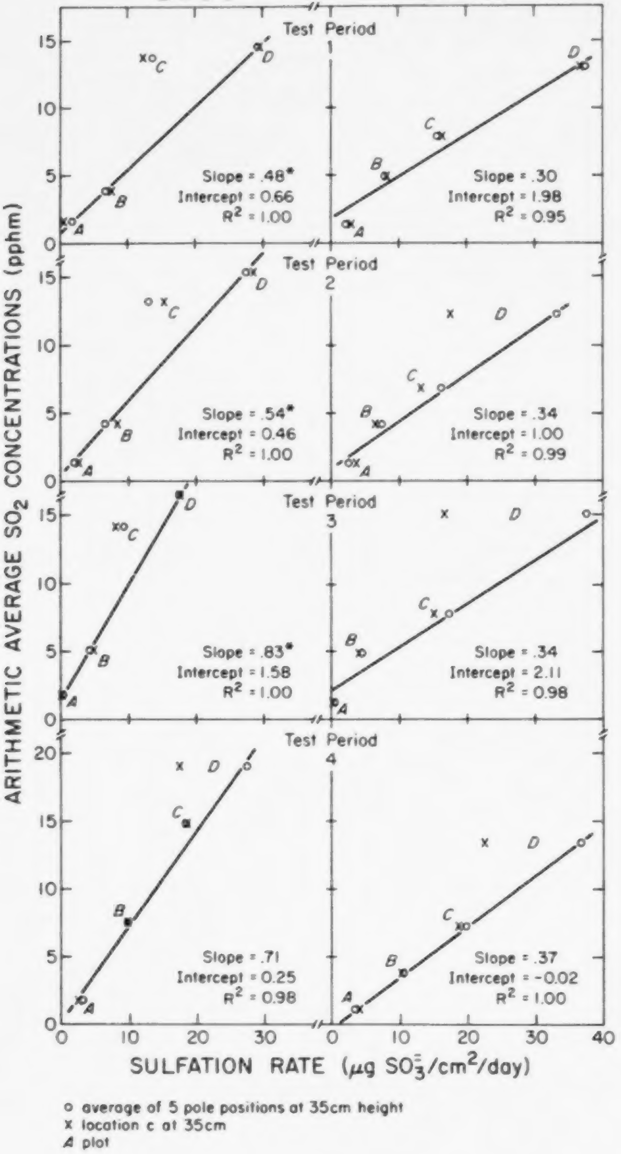


Figure 10.11. Relationship between sulfation rate and average SO_2 concentration during various time periods on the ZAPS plots.
* regression parameters based on data values for Plots A, B, and D only.

sulfation plates also serve as a check on performance of the real-time analyzers.

Figure 10.11 displays the relationship between mean SO_2 concentrations and sulfation rates on the plots for each test period. All measurements were made at 35 cm height. Mean SO_2 concentrations (ordinates) were estimated from the real-time monitoring data by the arithmetic average for ALL (high run and low run values, as described in Section 9) 7.5-minute median concentrations. Two estimates of sulfation rates (abscissas) are displayed. The first is the sulfation rate of the sulfation plate at pole positon 5 on each plot. The second is the mean sulfation rate for all 5 pole positions on each plot. The regression parameters shown, unless otherwise noted, are the least squares best linear fit between mean SO_2 concentrations and average sulfation rates at the five pole positions on each plot.

On ZAPS I, the regression lines are variable between test periods but they are nearly constant on ZAPS II. The slopes of the regression lines are steeper on ZAPS I than on ZAPS II. Sulfation rates tended to be lower on ZAPS I than on ZAPS II. The reasons for these differences between sites are unclear. Micrometeorological conditions at the two sites are quite similar (Section 11) and there is no reason to suspect that plates from the 2 sites were treated differently during analysis.

For ZAPS II, the conversion factor of

$$.35 \quad \frac{\text{pphm } SO_2}{\mu g\ SO_3/cm^2/day}$$

suggested by Corning Laboratories* appears to be reasonably accurate. However, the variability in conversion factors observed on ZAPS I suggests that these change in time and space. Assuming a constant conversion factor of

$$.35 \quad \frac{\text{pphm } SO_2}{\mu g\ SO_3/cm^2/day}$$

to estimate mean SO_2 concentrations from sulfation data could lead to underestimates greater than 2-fold.

For ZAPS I, Test periods 1, 2, and 3, the mean SO_2 concentrations for plot C are elevated considerably above those predicted by the linear regressions of points from the other three plots. The magnitude of this descrepancy decreases as the season progresses and seems to have essentially corrected itself by test period 4. The descrepancies are 89%, 75%, and 49% higher than expected for test periods 1, 2, and 3 respectively. This suggests a monitoring error in real-time measurements on ZAPS I, C during test periods 1, 2, and 3.

---

*mimeographed sheet supplied to sulfation plate users.

An additional indication that ZAPS I, C readings were artificially high comes from comparing these with readings on ZAPS II, C and ZAPS I, C taken during previous seasons. In all cases, the ZAPS I, C, 1977 readings appeared to be too high. Such elevated readings could be caused by a partially plugged sampling line leading from the C plot or any other mechanical problem in the time share sampling device increasing resistance to gas flow. On the basis of these indications, the ZAPS I, C, 1977 real-time readings reported in Section 9 (Table 9.2, Figure 9.4) have been reduced by the appropriate percentages for test periods 1, 2, and 3.

In most cases the sulfation rates at pole position 5 are similar to the mean sulfation rate for all five pole positions. This is inferred from the near coincidence of points in Figure 10.11. There are some exceptions to this on the D plots. On ZAPS I, D during test period 4 and on ZAPS II, D during test periods 2, 3, and 4, sulfation at pole position 5 is substantially lower than mean sulfation for the D plots. As previously shown, the distribution of sulfation rates on the D plots are non-random with greatest sulfation occurring at pole positions 1 and 4. This suggests that with the exception of the D plots where distributions of SO_2 in horizontal space are non-random, pole position 5 (location c) is a good place to monitor in order to estimate average concentrations in horizontal space on the plots.

Intensive Study

As in the EXTENSIVE study, vertical distribution of sulfation rates was examined through comparison of average sulfation values for the different heights (Table 10.4). Only data for the period 9/05/77 through 10/06/77 from poles within 2 m of the gas delivery pipes have been included in Table 10.4. This test period was used because it is the only one with any appreciable data for the 15 cm plates.

TABLE 10.4. AVERAGE SULFATION VALUES (AND STANDARD ERROR) FOR 15, 35, 75 AND 150 cm HEIGHTS, ZAPS I AND II, 9-05-77 → 10-06-77 EXPRESSED AS MICROGRAMS SULFATE AND AS A MULTIPLE OF 35 cm VALUE †

ZAPS	15 cm	35 cm	75 cm	150 cm
I	178.3(2.3)	277.8(5.6)	332.1(6.9)	211.6(2.2)
	0.64**	1	1.20**	0.76**
II	218.5(5.3)	358.0(8.7)	316.3(5.0)	201.2(3.6)
	0.61**	1	0.88**	0.56**

† Only poles within 2 m of delivery pipes are included

** = Significantly different at $P < .01$

ZAPS I, with the gas delivered horizontally from about 75 cm height above the ground, received more sulfur at the 75 cm level than at the 35 cm level. ZAPS II, with the downward discharge of gas from the less elevated delivery system, experienced greater sulfur at the 35 cm level than at the 75 cm level. Table 10.5 shows that these relationships hold when based on the combined values for three test periods.

TABLE 10.5. AVERAGE SULFATION VALUE (AND STANDARD ERROR) FOR 35, 75 AND 150 cm HEIGHTS, ZAPS I AND II, ALL TEST PERIODS COMBINED, EXPRESSED AS MICROGRAMS SULFATE AND AS A MULTIPLE OF 35 cm VALUE

ZAPS	35 cm	75 cm	150 cm
I	228.1(5.6)	281.0(6.9)	172.0(4.2)
	1	1.23**	0.75**
II	285.3(8.9)	254.0(8.3)	156.4(5.3)
	1	0.89*	0.55**

* = Significantly different at P <0.05

** = " " " P <0.01

The fine detail of horizontal distribution of fumigation gas was also examined with the INTENSIVE study. Positions were monitored 1, 2, 4, and 7 m from the gas delivery pipes. If the crossed fumigation pipes are seen as dividing the study area into four sections, (Figure 10.2) then the poles 1 m and 2 m from the pipes were sampling in three of the quadrants while the poles 4 and 7 m distant were sampling just in one. Only the 1 m and 2 m sulfation plates located in the southeasterly quadrant were used in comparing sulfation values at different horizontal distances from the delivery system. There were no statistically significant differences in the amounts of sulfur received at different distances from the pipes on either of the two ZAPS sites (Table 10.6). However, at 35 cm height on ZAPS II there was a reduction in sulfur concentration between the 1 m distance and the 2 m, 4 m, and 7 m ones. This may have been due to the slightly lower SO_2 delivery heights on ZAPS II. Here, SO_2 encounters less air turbulence and requires a slightly greater distance from the discharge orifice to reach stable dilution.

TABLE 10.6. AVERAGE SULFATION VALUES (AND STANDARD ERROR) FOR 35, 75 AND 150 cm PLATES, 1, 2, 4 AND 7 m DISTANT FROM DELIVERY PIPES, ALL TEST PERIODS COMBINED EXPRESSED AS MICROGRAMS SULFATE AND AS MULTIPLES OF 1 m DISTANCE

Height	1 m	2 m	4 m	7 m
ZAPS I				
35 cm	230.28(10.33)	220.24(11.84)	236.33(22.76)	228.33(21.26)
	1	0.96	1.03	0.99
75 cm	304.47(15.04)	268.90(13.84)	294.33(25.46)	264.00(30.62)
	1	0.88	0.97	0.87
150 cm	176.97(7.61)	168.10(9.27)	184.67(17.33)	179.33(22.64)
	1	0.95	1.04	1.01
ZAPS II				
35 cm	321.40(18.16)	273.38(15.86)	277.67(16.50)	258.00(16.07)
	1	0.85	0.86	0.80
75 cm	272.10(19.32)	256.62(16.01)	259.33(15.07)	255.00(21.36)
	1	0.94	0.95	0.94
150 cm	159.21(10.77)	168.05(9.09)	176.33(17.05)	181.67(11.89)
	1	1.06	1.11	1.14

Horizontal dispersion of sulfation rates are compared between ZAPS I and ZAPS II in Table 10.7. No significant difference exists at the 75 and 150 cm heights, but, ZAPS II, which apparently receives significantly more sulfur than ZAPS I at the 35 cm level, has a gradient of decreasing sulfur concentration with increasing distance from the gas delivery pipes.

CONCLUSIONS

Sulfation rates decrease with decreasing height above the ground within the plant canopy. The implied vertical profile of SO_2 concentrations is

TABLE 10.7. ZAPS II SULFATION VALUES (EXPRESSED AS MULTIPLES OF ZAPS I) FOR 35, 75 AND 150 cm HEIGHTS, 1, 2, 4, AND 7 m FROM DELIVERY PIPES

Distance from Pipe (m)	ZAPS II		
	35 cm	75 cm	150 cm
1	1.40**	0.89	0.90
2	1.24*	0.95	1.00
4	1.17	0.88	0.95
7	1.13	0.97	1.01

* $P < 0.05$

** $P < 0.01$

consistent with expectations derived from knowledge of atmospheric mixing processes and SO_2 removal potential within plant canopies. This implied vertical profile of SO_2 concentrations on the ZAPS plots is similar to that which might be expected within a grassland canopy experiencing fumigation from the plume of a coal-fired power plant.

Vertical stratification of SO_2 concentrations makes dose characterization complex. SO_2 exposure within the canopy depends upon the temporal pattern in which constituent organisms make use of vertical and horizontal space.

Prevailing wind patterns cause drift of SO_2 onto the A plots at both ZAPS sites. Seasonal averages of SO_2 concentrations on these plots are roughly one fourth of the concentrations on the B-plots. While SO_2 concentrations vary considerably in horizontal space at canopy height, only the D-plots have statistically significantly different mean concentrations between positions monitored. The pattern of sulfation rates shown on the D plots correlates well with expectations derived from the limited available knowledge of wind patterns on the plots.

Mean concentrations of SO_2 at 35 cm height on the plots are in direct proportion to the delivery rates of SO_2 to the plots.

Conversion factors relating sulfation rates to SO_2 concentrations are variable in time and space. Errors in estimates of 2 to 3 fold could be expected by assuming a constant conversion factor throughout the season.

The Intensive Study suggests that mixing occurs fairly rapidly with distance from the gas delivery orifices. On ZAPS I, stable SO_2 dilution appears to be reached within about one meter of the gas delivery orifices.

On ZAPS II, stable dilution may not be reached until two meters from the orifices.

REFERENCES

Inoue, E. 1963. On the Turbulent Structure of Airflow within Crop Canopies. J. Meteorol. Soc. Japan, 41(6):317-325.

Liang, S.F., C.V. Sternling, and T.R. Galloway. 1973. Evaluation of the Effectiveness of the Lead Peroxide Method for Atmospheric Monitoring of Sulfur Dioxide. J. Air Poll. Cont. Assoc., 23(7):605-607.

SECTION 11

SOIL AND METEOROLOGICAL CHARACTERISTICS AT ZAPS

J. L. Dodd, W. K. Lauenroth, R. G. Woodmansee,
G. L. Thor and J. D. Chilgren

SOIL DESCRIPTION: PHYSICAL AND CHEMICAL TRAITS

Soils of both ZAPS sites have been characterized by standard soil descriptions (R. G. Woodmansee) and extensive physical and chemical analyses. Three soil pits were excavated on ZAPS I and five on ZAPS II. The ZAPS I pits were located between the control and low plots (west pit), between the low and medium plots (middle pit), and adjacent to the northwest edge of the control plot (northwest pit). The ZAPS II pits were located immediately west of the control, between the control and low plots, between the low and medium plots, between the medium and high plots, and immediately east of the high plot.

Soils at ZAPS I were classified as Farland silty clay loams (Table 11.1) and at ZAPS II as Thurlow clay loams (Table 11.2). Physical and chemical characteristics of the soil pits are presented in Tables 11.3 to 11.8.

Soils of the study sites possess substantial within-site variability in physical and chemical characteristics because of variance in both parent material and micro-topographic position, which is common on native rangelands.

SOIL WATER DYNAMICS AND PRECIPITATION

Soil water dynamics for ZAPS I and II were determined by gravimetric sampling. Summaries of soil water dynamics on the ZAPS sites are shown in Figures 11.1 and 11.2. A more detailed account appears in Tables 11.9 to 11.13. In general, soil water content was maintained at much higher levels in 1975 and 1976 than in 1977. This was partly because of lower precipitation during the 1977 growing season (Table 11.14) and apparently lower evapotranspiration rates in the early part of the 1975 and 1976 seasons (Section 13). Precipitation was greatest in May and June at both ZAPS in 1976 and 1977. July was particularly arid in 1977. On individual days, precipitation was characteristically in quantities of 0.25-15 mm, the mode being 1-5 mm both years (Figures 11.3 and 11.4).

TABLE 11.1. PROFILE DESCRIPTION FOR ZAPS I. FARLAND SILTY CLAY LOAM SOIL SERIES, WEST PIT

Horizon	Depth (cm)	Description
A11	0-15	Brown (10 YR 5/3); very dark brown (10 YR 3/3); clay loam; massive; soft, friable, nonsticky and slightly plastic; noneffervescent; medium acid (pH 5.8); clear smooth boundary; many fine roots; few fine pores.
A12	15-25	Gray brown (2.5 Y 5/2); dark brown (10 YR 4/3); silt loam; weak massive and columnar blocky; soft, very friable, nonsticky, slightly plastic; noneffervescent; slightly acid (pH 6.1); clear wavy; many fine roots; many fine pores.
B21	25-39	Yellowish brown (10 YR 5/4); yellowish brown crushed (10 YR 5/6); dark brown coats (10 YR 4/4); silty clay loam; weak columnar prisms parting to weak massive blocky; hard, very firm, sticky, plastic; noneffervescent; medium acid (pH 5.9); clear wavy; common fine roots; many fine pores.
B22	39-79	Light olive brown (2.5 Y 5/4); yellowish brown crushed (10 YR 5/4); dark brown coats (10 YR 4/3); clay loam; strong coarse prisms parting to moderate coarse blocky; very hard, extremely firm, very sticky, very plastic; noneffervescent; neutral (pH 7.0); clear wavy; common fine roots; few fine pores.
B3ca	79-91	Light olive brown (2.5 Y 5/4); light olive brown crushed (2.5 Y 5/4); olive brown coats (2.5 Y 4/4); clay loam; weak coarse prisms; very hard, extremely firm, very sticky, very plastic; slightly effervescent; moderately alkaline (pH 7.9), threads and very small pockets (1 mm); gradual wavy; few fine roots; many fine pores.
C1ca	91-134	Light olive brown (2.5 Y 5/4); olive brown (2.5 Y 4/4); clay loam; massive; very hard, extremely firm, very sticky, very plastic; slightly effervescent, moderately alkaline (pH 8.0), small pockets (2.3 mm); clear wavy; very few fine roots; many fine pores.
C2ca	134-	Light olive brown (2.5 Y 5.5/4); light olive brown bulk (2.5 Y 5/4), pale yellow lime streaks (2.5 Y 8/4); clay loam; massive; hard, firm, very sticky, very plastic; slightly effervescent; moderately alkaline (pH 8.0), large pockets (7 mm); not reached boundary.

Remarks: The A1 thickness ranges 15-25 cm, solum depth 90-105 cm, thickness of B 66-75.

TABLE 11.2. PROFILE DESCRIPTIONS FOR ZAPS II. THURLOW CLAY LOAM SOIL SERIES, PIT WEST OF CONTROL

Horizon	Depth (cm)	Description
A11	0–2	Grayish brown (10 YR 5.5/2) dry; very dark grayish brown (10 YR 3/2) moist; loam; structureless; loose, nonsticky, nonplastic; noneffervescent; slightly acid (pH 6.1); abrupt wavy boundary; many fine roots.
A12	2–12	Dark brown (10 YR 4/3) dry and moist; clay loam; thin platy; firm, hard, slightly sticky and slightly plastic to plastic; noneffervescent; slightly acid (pH 6.4); clear wavy boundary; many fine roots.
B21	12–30	Yellowish brown (10 YR 5/4) dry; dark brown (10 YR 4/3) moist; clay loam; moderate, medium prisms breaking to strong medium and coarse subangular blocky structure; hard, firm, sticky, and slightly plastic to plastic; noneffervescent; neutral (pH 7.0); clear, wavy boundary; many fine roots.
B22	30–44	Brown crushed (10 YR 5/3) dry; dark brown coats (10 YR 3.5/3) dry; dark brown crushed (10 YR 4/3) moist; very dark grayish brown coats (10 YR 3/2) moist; clay loam; strong coarse prisms breaking to strong very coarse subangular blocky structure; very hard, very firm, sticky and plastic; noneffervescent; neutral (pH 7.3); clear, wavy boundary; many fine roots.
B23ca	44–80	Yellowish brown coats (10 YR 5/4) dry, peds too hard to crush; dark brown coats (10 YR 4/3) moist, peds too hard to crush; clay loam; very strong medium prisms breaking to very strong medium subangular blocky structure; very hard, extremely firm, sticky and slightly plastic to plastic; effervescent, pockets and riffins of lime; moderately alkaline (pH 8.1); clear, wavy boundary; common fine roots.
B3	80–100	Yellowish brown coats (10 YR 5/4) with patches of dark brown (10 YR 4/3) and very dark grayish brown (10 YR 3/2) dry, peds too hard to crush; dark brown coats (10 YR 3/3) moist; clay loam; weak medium prisms breaking to moderate medium subangular blocky structure; very hard, extremely firm, slightly sticky to sticky and plastic; noneffervescent (sic), a few fine wires of lime present but not enough to cause effervescence; moderately alkaline (pH 7.9); pockets of iron and gypsium; diffuse, wavy boundary; common fine roots.
C1	100–152	Brown (10 YR 5/3) dry; dark brown (10 YR 4/3) moist; clay loam; structureless; very hard, very firm, slightly sticky and slightly plastic to plastic; effervescent, lime in small pockets; moderately alkaline (pH 8.0); few fine roots.
Remarks:	This pit is probably a representative Thurlow profile and lies at the west end of the ZAPS II site. The soils to the east of this pit are underlain first (moving eastward) by one buried profile and at the eastern end of the site two buried profiles are represented. However, the current, pedogenic profiles of these soils are similar to the profile described herein. The depth of the Thurlow solum ranges from 100 cm (described here) to 28 cm however some current pedogenic activity is apparent in the shallower buried A horizons.	

TABLE 11.3. PHYSICAL CHARACTERISTICS OF ZAPS I SITE SOILS

Sample number	Horizon	Depth (cm)	Gravel (%)	Sand (%)	Silt (%)	Clay (%)	Texture*	Bulk density
			ZAPS I, West Pit					
322P	A11	0-15	1.96	61	22	17	SL	
324P	A12	15-25	1.85	61	23	16	SL	1.41
325P	B21	25-39	1.50	50	23	27	SCL	1.57
326P	B22	39-79	0.17	35	25	40	CL	1.62
327P	B3ca	79-91	0.10	43	24	33	CL	
328P	C1ca	91-134	1.89	41	23	36	CL	
329P	C2ca	134±	2.37	39	25	36	CL	
			ZAPS I, Middle Pit					
323P	A11	0-9	2.12	41	22	27	Cl/L	1.26
405P	A11	0-9	2.12	43	29	28	CL	1.26
336P	A12	9-15	3.23	44	23	33	CL	1.33
337P	A3	15-32	1.82	39	26	35	CL	1.32
406P	A3	15-32	1.82	39	24	37	CL	1.32
338P	B21	32-49	1.99	33	22	45	CL	1.53
339P	B22	49-67	4.03	29	23	48	C	1.57
340P	B23ca	67-86	0.30	35	22	43	C	
341P	B3ca	86-104	0.23	36	21	43	C	
			ZAPS I, Northwest Pit					
	A11	0-2		52	29	19	SL	
	A12	2-24		38	32	30	CL	
	B2	24-77		46	16	38	SCL	
	B3	77-92		41	18	41	C	
	C	92+		36	22	42	C	

*C = clay, L - loam, Si = silt, S = sand.

TABLE 11.4. WATER-HOLDING CAPACITY OF ZAPS I SITE SOILS*

Sample number	Horizon	Depth (cm)	0 bar		0.33 bar		1 bar		15 bar	
ZAPS I, West Pit										
322P	A11	0-15	38.32	38.23	14.40	12.00	9.75	8.75	4.19	4.40
324P	A12	15-25	36.37	38.41	11.50	12.30	7.73	7.57	4.28	4.52
325P	B21	25-39	47.48	47.27	12.45	13.69	9.96	9.51	3.94	4.01
326P	B22	39-79	61.34	61.04	20.72	24.41	17.43	18.04	11.26	10.79
327P	B3ca	79-91	55.64	59.01	17.38	16.98	14.76	14.95	9.02	9.51
328P	C1ca	91-134	54.47	48.96	22.45	20.91	19.01	16.62	9.67	9.75
329P	C2ca	134+	53.48	53.77	23.04	21.06	15.63	15.81	9.66	9.20
ZAPS I, Middle Pit										
323P	A11	0-9	50.14	50.87	19.47	20.34	14.36	13.66	6.58	6.46
405P	A11	0-9								
336P	A12	9-15	61.31	50.44	18.18	17.71	11.53	13.53	8.29	7.56
337P	A3	15-32	49.87	49.81	20.88	18.42	15.24	15.32	9.00	9.01
406P	A3	15-32								
338P	B21	32-49	59.18	56.35	25.62	23.92	19.05	19.35	12.06	11.58
339P	B22	49-67	65.66	61.19	16.02	25.49	20.62	17.96	12.90	13.12
340P	B23ca	67-86	59.96	56.25	22.79	21.57	18.06	18.37	11.55	11.39
341P	B3ca	86-104	56.88	56.69	22.94	23.17	18.05	17.91	11.31	10.87

---

* % wt.

TABLE 11.5. CHEMICAL CONSTITUENTS OF ZAPS I SITE SOILS

Sample number	Horizon	Depth (cm)	Organic matter	pH	Lime (%)	N (%)	Total P (%)	Inorganic P (%)	Bicarbonate P (%)
				ZAPS I, West Pit					
322P	A11	0-15	1.2	6.1	0.0	0.078	0.030	0.017	1
324P	A12	15-25	0.6	6.1	0.0	0.043	0.028	0.020	4
325P	B21	25-39	1.0	5.9	0.0	0.081	0.035	0.021	1
326P	B22	39-79	0.6	7.0	0.0	0.049	0.041	0.032	1
327P	B3ca	79-91	0.4	7.9	1.2	0.030	0.035	0.030	1
328P	C1ca	91-134	0.4	8.0	3.0	0.030	0.034	0.030	1
329P	C2ca	134+	0.4	8.0	2.8	0.031	0.038	0.035	4
				ZAPS I, Middle Pit					
323P	A11	0-9	1.8	5.8	0.0	0.119	0.034	0.020	2
405P	A11	0-9	2.2	5.7	0.0	0.128	0.036	0.023	1
336P	A12	9-15	1.3	6.1	0.0	0.089	0.030	0.016	5
337P	A3	15-32	1.0	6.2	0.0	0.079	0.030	0.016	1
406P	A3	15-32	1.2	6.2	0.0	0.081	0.030	0.016	2
338P	B21	32-49	1.1	6.8	0.0	0.078	0.038	0.025	3
339P	B22	49-67	1.0	7.1	0.0	0.077	0.045	0.031	2
340P	B23ca	67-86	0.9	7.7	1.0	0.065	0.043	0.031	1
341P	B3ca	86-104	0.9	7.9	0.8	0.059	0.045	0.033	1
				ZAPS I, Northwest Pit					
	A11	0-2	4.0	6.1	0.0	0.275	0.051	0.021	12
	A12	2-24	1.6	5.8	0.0	0.103	0.052	0.034	1
	B2	24-77	0.8	7.3	0.5	0.070	0.040	0.030	1
	B3	77-92	0.6	7.8	0.8	0.046	0.037	0.028	1
	C	92+	0.5	8.1	1.8	0.040	0.040	0.035	1

TABLE 11.6. EXCHANGEABLE IONS OF ZAPS I SITE SOILS [*]

Sample number	Horizon	Depth (cm)	SO_4	CEC	CA	Mg	Na	K	K (ppm)
				ZAPS I, West Pit					
322P	A11	0-15	0.1	7.2	4.4	1.8	<0.1	0.4	165
324P	A12	15-25	2.0	6.7	3.1	2.2	<0.1	0.3	100
325P	B21	25-39	0.6	14.2	4.7	6.5	0.1	0.4	165
326P	B22	39-79	<0.1	24.6	9.4	14.5	0.2	0.3	125
327P	B3ca	79-91	<0.1	17.3	--	--	0.2	0.3	105
328P	C1ca	91-134	0.7	17.3	--	--	0.3	0.3	120
329P	C2ca	134+	1.4	17.8	--	--	0.4	0.3	110
				ZAPS I, Middle Pit					
323P	A11	0-9	0.5	13.8	7.8	2.8	<0.1	0.5	200
405P	A11	0-9	1.4	13.6	7.3	3.2	0.1	0.6	225
336P	A12	9-15	0.3	14.2	7.5	4.4	0.1	0.4	175
337P	A3	15-32	0.6	14.6	6.5	5.5	0.1	0.3	135
406P	A3	15-32	1.9	19.8	8.9	6.6	0.1	0.4	145
338P	B21	32-49	<0.1	21.8	9.3	10.2	0.3	0.4	140
339P	B22	49-67	1.7	26.6	10.5	14.4	0.6	0.4	165
340P	B23ca	67-86	<0.1	21.9	--	--	0.7	0.4	140
341P	B3ca	86-104	<0.1	19.7	--	--	0.8	0.4	140

[*] meq · 100 g^{-1} soil.

TABLE 11.7. PHYSICAL CHARACTERISTICS OF ZAPS II SITE SOILS

Horizon	Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Texture*
ZAPS II, Pit West of Control					
A11	0-2	34	41	25	L
A12	2-12	32	34	34	CL
B21	12-30	34	33	33	CL
B22	30-44	43	28	29	CL
B23ca	44-80	37	28	35	CL
B3	80-100	43	27	30	CL
C1	100+	38	32	30	CL
ZAPS II, Control-Low Pit					
A11	0-3	36	39	25	L
A12	3-13	32	34	34	CL
B21	13-29	27	34	39	CL
B22	29-47	34	32	34	CL
IIA1	47-63	30	27	43	C
IIB2	63-75	28	27	45	C
IIIA1	75-112	27	32	41	C
IIIB2	112-125	36	28	36	CL
C1	125+	38	30	32	CL
ZAPS II, Low-Medium Pit					
A11	0-2	29	35	36	CL
A12	2-11	25	33	42	C
B2	11-40	26	35	39	CL
IIA1	40-56	30	33	37	CL
IIB2	56-88	26	29	45	C
IIB3	88-130	30	33	37	CL
IIC1	130+	33	32	35	CL
ZAPS II, Medium-High Pit					
A11	0-3	28	40	32	CL
A12	3-11	27	32	41	C
B2	11-28	--	--	--	--
IIA1	28-43	22	35	43	C
IIB21	43-65	22	36	42	C
IIB22	65-79	25	39	36	CL
IIB3	79-118	20	39	41	C
IIC	118+	24	36	40	C
ZAPS II, Pit East of High					
A11	0-3	24	40	36	CL
A12	3-11	24	40	36	CL
B21	11-24	20	45	35	CL
B22	24-40	26	42	32	CL
IIB23	40-73	20	44	36	CL
IIB24	73-119	32	33	35	CL
IIIA1	119-136	22	37	41	C
IIIB2	136-152	26	37	37	CL

*C = clay, L = loam, Si = silt, S = sand.

TABLE 11.8. CHEMICAL CONSTITUENTS OF ZAPS II SITE SOILS

Horizon	Depth (cm)	Organic matter (%)	pH	Lime (%)	N (%)	Total P (%)	Inorganic P (%)	Bicarbonate P (%)
ZAPS II, Pit West of Control								
A11	0-2	5.8	6.1	<0.1	0.319	0.074	0.044	10
A12	2-12	2.5	6.4	<0.1	0.153	0.055	0.040	2
B21	12-30	1.5	7.0	<0.1	0.119	0.053	0.040	1
B22	30-44	1.1	7.3	0.7	0.090	0.048	0.036	1
B23ca	44-80	1.0	8.1	1.2	0.083	0.045	0.037	1
B3	80-100	1.2	7.9	1.8	0.082	0.057	0.043	1
C1	100+	1.4	8.0	4.8	0.080	0.060	0.047	3
ZAPS II, Control-Low Pit								
A11	0-3	4.0	6.3	<0.1	0.261	0.068	0.040	13
A12	3-13	1.3	6.6	<0.1	0.102	0.049	0.040	2
B21	13-29	1.6	7.7	0.8	0.107	0.054	0.038	1
B22	29-47	0.9	7.8	1.2	0.074	0.048	0.040	1
IIA1	47-63	1.0	7.7	1.1	0.075	0.038	0.024	1
IIB2	63-75	1.3	7.8	1.1	0.098	0.045	0.032	1
IIA1	75-112	3.2	7.8	2.0	0.187	0.061	0.040	7
IIB2	112-125	1.4	8.3	5.4	0.089	0.057	0.043	2
C1	125+	0.8	8.3	4.7	0.061	0.057	0.049	2
ZAPS II, Low-Medium Pit								
A11	0-2	0.7	7.9	4.7	0.054	0.057	0.044	1
A12	2-11	1.8	6.6	<0.1	0.143	0.055	0.041	1
B2	11-40	1.3	7.4	1.2	0.085	0.058	0.043	1
IIA1	40-56	1.4	7.9	1.1	0.095	0.049	0.035	1
IIB2	56-88	1.1	7.8	1.4	0.075	0.051	0.041	1
IIB3	88-130	0.9	8.4	4.7	0.066	0.049	0.039	1
IIC1	130+	0.8	8.4	8.2	0.052	0.054	0.045	4
ZAPS II, Medium-High Pit								
A11	0-3	4.2	6.8	<0.1	0.261	0.071	0.044	10
A12	3-11	1.9	6.9	<0.1	0.142	0.059	0.043	1
B2	11-28	--	--	--	--	--	--	--
IIA1	28-43	3.0	7.8	2.3	0.154	0.059	0.040	2
IIB21	43-65	2.0	7.8	2.9	0.120	0.061	0.044	1
IIB22	65-79	1.3	7.9	2.4	0.085	0.060	0.050	1
IIB3	79-118	1.7	8.4	3.7	0.109	0.065	0.048	1
IIC	118+	1.3	8.4	5.2	0.065	0.061	0.047	2
ZAPS II, Pit East of High								
A11	0-3	4.8	7.0	<0.1	0.285	0.076	0.043	3
A12	3-11	2.0	7.2	<0.1	0.128	0.061	0.049	1
B21	11-24	1.7	7.8	2.6	0.110	0.060	0.045	1
B22	24-40	5.4	6.6	<0.1	0.327	0.079	0.048	8
IIB23	40-73	1.9	7.6	3.2	0.123	0.065	0.050	1
IIB24	73-119	0.9	7.8	1.4	0.072	0.059	0.047	1
IIIA1	119-136	1.0	7.8	2.1	0.070	0.059	0.046	1
IIIB2	136-152	2.4	8.1	3.4	0.115	0.058	0.041	1

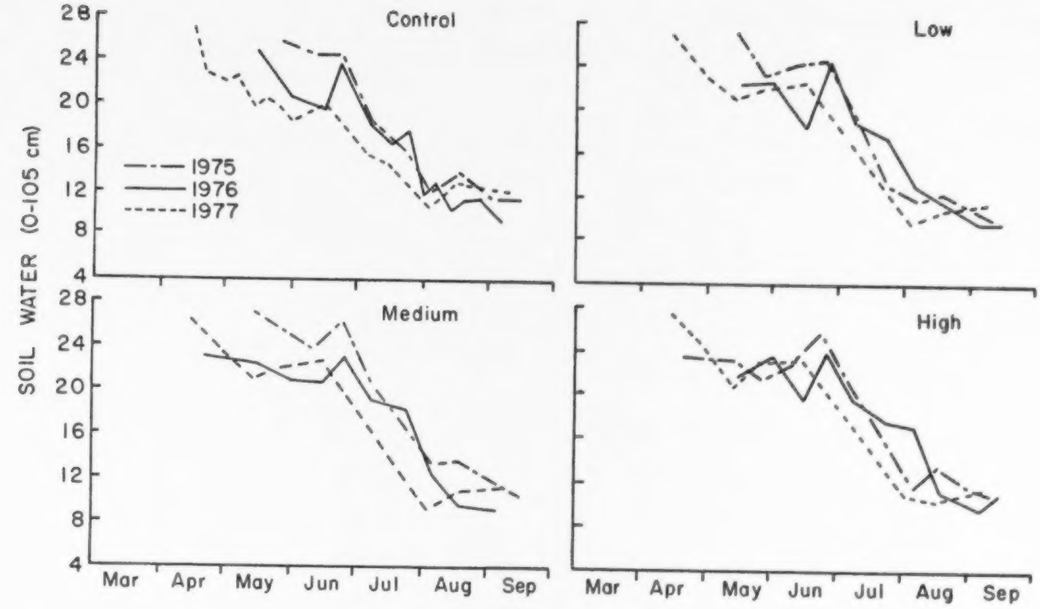


Figure 11.1. Seasonal dynamics of soil water (cm) for ZAPS I, 1975-1977.

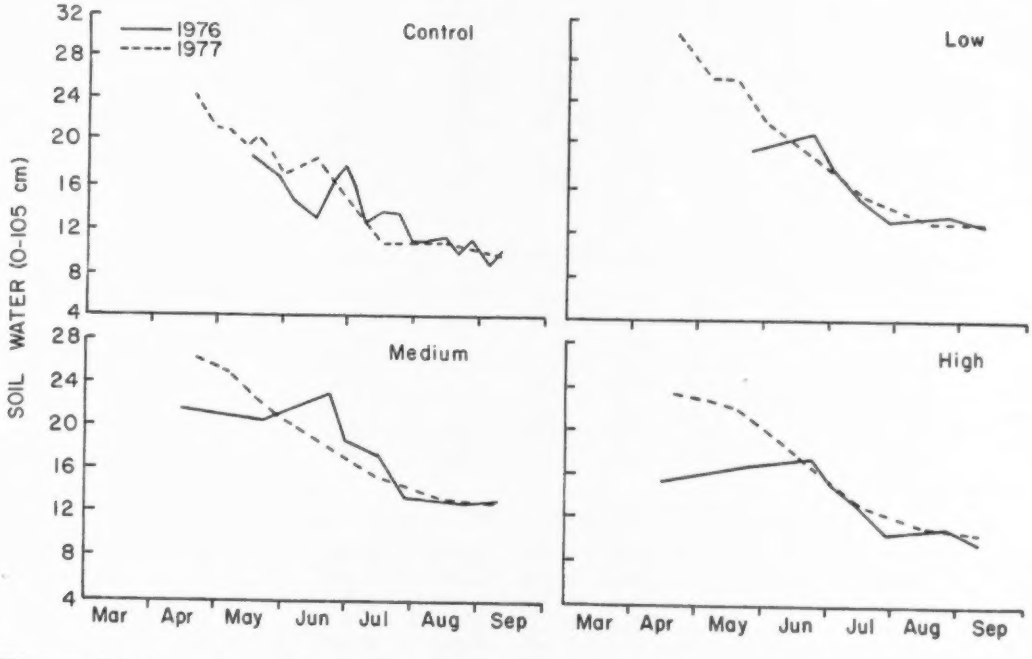


Figure 11.2. Seasonal dynamics of soil water (cm) for ZAPS II, 1976-1977.

TABLE 11.9. SEASONAL DYNAMICS OF SOIL WATER, ZAPS I, 1975*

Treatment	Depth (cm)	12 May	26 May	10 June	22 June	7 July	21 July	5 August	18 August	2 September	15 September
Control	0-15		3.53 ± 0.18	3.57 ± 0.14	3.84 ± 0.18	1.38 ± 0.06	1.20 ± 0.07	0.58 ± 0.01	1.33 ± 0.18	0.83 ± 0.04	0.43 ± 0.05
	15-30		3.51 ± 0.09	3.12 ± 0.04	3.71 ± 0.12	2.07 ± 0.13	1.46 ± 0.13	0.97 ± 0.13	1.37 ± 0.05	1.34 ± 0.19	1.19 ± 0.07
	30-45		4.13 ± 0.19	4.32 ± 0.21	4.00 ± 0.09	2.83 ± 0.36	1.98 ± 0.02	1.71 ± 0.14	1.98 ± 0.09	1.72 ± 0.03	1.88 ± 0.30
	45-60		4.28 ± 0.27	4.13 ± 0.40	4.01 ± 0.13	3.45 ± 0.44	2.68 ± 0.05	1.72 ± 0.20	2.09 ± 0.29	1.87 ± 0.28	1.86 ± 0.30
	60-75		3.66 ± 0.15	3.73 ± 0.03	3.49 ± 0.24	3.04 ± 0.27	2.86 ± 0.25	1.94 ± 0.11	2.07 ± 0.17	1.64 ± 0.56	1.80 ± 0.13
	75-90		3.60 ± 0.30	3.14 ± 0.13	3.15 ± 0.47	2.94 ± 0.09	2.77 ± 0.36	2.55 ± 0.07	2.33 ± 0.06	2.15 ± 0.09	2.09 ± 0.08
	90-105		3.08 ± 0.49	2.84 ± 0.10	2.77 ± 0.35	2.76 ± 0.07	2.62 ± 0.37	2.61 ± 0.28	2.64 ± 0.23	2.15 ± 0.03	2.26 ± 0.08
	TOTAL		25.68	24.85	24.98	18.47	15.57	12.08	13.81	11.71	11.51
Low	0-15	3.73 ± 0.07	3.37 ± 0.03	3.24 ± 0.09	3.66 ± 0.10	1.16 ± 0.15	0.65 ± 0.05	0.58 ± 0.04	1.15 ± 0.05	0.72 ± 0.10	0.43 ± 0.03
	15-30	3.73 ± 0.16	3.49 ± 0.12	3.19 ± 0.14	3.54 ± 0.14	2.36 ± 0.15	0.95 ± 0.20	1.05 ± 0.09	1.28 ± 0.13	1.02 ± 0.08	1.01 ± 0.07
	30-45	4.03 ± 0.12	3.73 ± 0.11	3.93 ± 0.09	3.88 ± 0.10	3.38 ± 0.15	2.11 ± 0.08	1.54 ± 0.13	1.72 ± 0.09	1.59 ± 0.03	1.55 ± 0.11
	45-60	4.49 ± 0.17	4.07 ± 0.13	4.33 ± 0.13	4.15 ± 0.15	3.67 ± 0.38	2.65 ± 0.24	1.94 ± 0.11	2.11 ± 0.07	1.94 ± 0.08	1.77 ± 0.10
	60-75	3.73 ± 0.27	3.27 ± 0.32	3.67 ± 0.11	3.72 ± 0.02	3.08 ± 0.27	2.58 ± 0.05	1.90 ± 0.13	1.93 ± 0.14	1.93 ± 0.10	1.54 ± 0.07
	75-90	3.58 ± 0.27	2.54 ± 0.16	3.18 ± 0.17	3.11 ± 0.17	2.85 ± 0.26	2.37 ± 0.09	2.14 ± 0.24	1.99 ± 0.22	2.02 ± 0.20	1.66 ± 0.12
	90-105	3.31 ± 0.20	2.48 ± 0.07	2.56 ± 0.13	2.64 ± 0.48	2.76 ± 0.27	2.18 ± 0.08	2.81 ± 0.69	2.02 ± 0.20	1.62 ± 0.36	1.71 ± 0.15
	TOTAL	26.60	22.95	24.10	24.71	19.25	13.49	11.96	12.21	10.85	9.67
Medium	0-15	3.68 ± 0.34	3.33 ± 0.16	3.28 ± 0.25	3.51 ± 0.12	1.39 ± 0.15	0.98 ± 0.16	0.86 ± 0.08	1.37 ± 0.13	0.89 ± 0.09	0.65 ± 0.11
	15-30	3.69 ± 0.22	3.59 ± 0.15	3.27 ± 0.15	3.65 ± 0.16	2.78 ± 0.17	1.45 ± 0.09	1.94 ± 0.51	1.66 ± 0.11	1.43 ± 0.05	1.30 ± 0.11
	30-45	4.16 ± 0.17	4.53 ± 0.61	3.69 ± 0.42	4.21 ± 0.18	3.27 ± 0.12	2.39 ± 0.04	1.90 ± 0.18	2.08 ± 0.10	1.79 ± 0.08	1.71 ± 0.04
	45-60	4.18 ± 0.23	3.76 ± 0.19	3.75 ± 0.22	3.92 ± 0.14	3.30 ± 0.08	2.82 ± 0.10	2.17 ± 0.17	2.03 ± 0.14	1.88 ± 0.02	1.59 ± 0.04
	60-75	3.96 ± 0.22	3.62 ± 0.03	3.58 ± 0.06	3.71 ± 0.17	3.28 ± 0.08	2.95 ± 0.06	1.91 ± 0.09	2.08 ± 0.09	1.76 ± 0.03	1.61 ± 0.01
	75-90	3.62 ± 0.09	3.57 ± 0.05	3.55 ± 0.07	3.62 ± 0.09	3.43 ± 0.11	3.17 ± 0.01	2.12 ± 0.10	2.16 ± 0.11	1.96 ± 0.15	1.72 ± 0.07
	90-105	3.87 ± 0.02	3.46 ± 0.06	2.86 ± 0.06	3.78 ± 0.19	3.30 ± 0.03	2.65 ± 0.40	2.40 ± 0.30	2.39 ± 0.08	2.35 ± 0.35	1.80 ± 0.19
	TOTAL	27.15	25.86	23.99	26.41	20.76	16.40	13.30	13.76	12.05	10.37
High	0-15	3.89 ± 0.11	3.60 ± 0.20	3.58 ± 0.18	4.04 ± 0.11	1.59 ± 0.12	1.04 ± 0.10	0.88 ± 0.04	1.12 ± 0.09	0.75 ± 0.25	0.76 ± 0.05
	15-30	3.73 ± 0.03	3.25 ± 0.37	3.32 ± 0.04	3.87 ± 0.17	2.68 ± 0.38	1.40 ± 0.15	1.16 ± 0.14	1.39 ± 0.11	1.34 ± 0.10	1.22 ± 0.14
	30-45	3.83 ± 0.22	4.12 ± 1.15	3.79 ± 0.22	3.75 ± 0.13	3.03 ± 0.10	1.97 ± 0.08	1.82 ± 0.11	1.78 ± 0.09	1.90 ± 0.10	1.74 ± 0.03
	45-60	3.60 ± 0.66	3.17 ± 0.50	3.77 ± 0.23	4.02 ± 0.17	3.52 ± 0.06	2.82 ± 0.13	2.08 ± 0.22	2.21 ± 0.18	1.52 ± 0.56	1.90 ± 0.08
	60-75	2.81 ± 0.68	2.47 ± 0.60	3.21 ± 0.36	3.46 ± 0.30	3.43 ± 0.01	2.77 ± 0.07	1.94 ± 0.19	2.68 ± 0.65	2.01 ± 0.26	1.87 ± 0.07
	75-90	2.45 ± 0.36	2.55 ± 0.52	2.97 ± 0.43	3.42 ± 0.50	2.79 ± 0.48	2.71 ± 0.05	1.84 ± 0.40	1.98 ± 0.01	1.85 ± 0.13	1.86 ± 0.08
	90-105	2.83 ± 0.48	2.58 ± 0.37	2.46 ± 0.71	3.17 ± 0.48	3.08 ± 0.26	3.40 ± 0.83	2.03 ± 0.15	2.09 ± 0.07	1.99 ± 0.32	1.83 ± 0.20
	TOTAL	23.14	21.74	23.11	25.73	20.11	16.11	11.75	13.36	11.37	11.19

---

* cm, $\bar{X}$ ± SE.

TABLE 11.10. SEASONAL DYNAMICS OF SOIL WATER, ZAPS I, 1976*

Treatment	Depth (cm)	20 March	14 April	20 April	16 May	1 June	13 June	21 June	24 June	1 July
Control	0-15	3.55 ± 0.04	3.48 ± 0.13	3.78 ± 0.19	3.36 ± 0.16	3.09 ± 0.10	2.62 ± 0.19	3.49 ± 0.17	3.63 ± 0.24	2.46 ± 0.25
	15-30	3.21 ± 0.14	3.05 ± 0.17	3.80 ± 0.05	2.98 ± 0.22	3.52 ± 0.23	2.54 ± 0.27	3.27 ± 0.28	2.99 ± 0.38	3.12 ± 0.11
	30-45	3.35 ± 0.36	3.61 ± 0.82	3.56 ± 0.72	4.45 ± 0.27	3.91 ± 0.29	3.21 ± 0.25	3.61 ± 0.55	3.35 ± 0.61	3.04 ± 0.84
	45-60	2.69 ± 0.40	2.96 ± 1.01	3.04 ± 0.95	2.91 ± 0.33	2.47 ± 0.21	2.92 ± 0.46	3.82 ± 0.67	3.10 ± 0.56	3.23 ± 0.47
	60-75		3.90 ± 0.00	2.86 ± 0.58	3.99 ± 1.05	2.83 ± 0.73	2.70 ± 0.41	3.20 ± 0.66	2.96 ± 0.56	2.82 ± 0.32
	75-90		2.42 ± 0.00	2.94 ± 0.37	3.47 ± 0.38	2.32 ± 0.15	2.72 ± 0.44	3.39 ± 0.75	2.69 ± 0.45	2.65 ± 0.31
	90-105				3.44 ± 1.11	2.54 ± 0.25	2.59 ± 0.34	2.98 ± 0.40	2.65 ± 0.34	2.68 ± 0.45
	TOTAL				24.58	20.67	19.31	23.75	21.36	20.01
Low	0-15	3.64 ± 0.23		3.63 ± 0.09	3.26 ± 0.05	3.29 ± 0.07	2.38 ± 0.13		3.45 ± 0.11	
	15-30	3.27 ± 0.51		3.60 ± 0.02	3.36 ± 0.11	3.32 ± 0.04	2.37 ± 0.07		3.55 ± 0.07	
	30-45	3.42 ± 0.95		3.92 ± 0.24	3.87 ± 0.07	3.83 ± 0.17	3.05 ± 0.15		4.13 ± 0.39	
	45-60	3.01 ± 0.00		3.30 ± 0.46	3.86 ± 0.32	4.17 ± 0.19	3.27 ± 0.20		3.98 ± 0.58	
	60-75	2.19 ± 0.00		2.04 ± 0.08	3.05 ± 0.17	3.26 ± 0.19	2.58 ± 0.14		3.49 ± 0.17	
	75-90			1.89 ± 0.06	2.75 ± 0.10	2.67 ± 0.30	2.44 ± 0.02		2.67 ± 0.29	
	90-105				2.05 ± 0.42	1.96 ± 0.12	2.25 ± 0.17		3.28 ± 0.56	
	TOTAL				22.19	22.50	18.34		24.55	
Medium	0-15	3.57 ± 0.08		3.61 ± 0.03	3.38 ± 0.19	3.10 ± 0.19	2.45 ± 0.18		3.38 ± 0.11	
	15-30	2.82 ± 0.50		3.66 ± 0.19	3.38 ± 0.30	3.75 ± 0.26	2.65 ± 0.14		3.44 ± 0.18	
	30-45	3.14 ± 0.05		3.72 ± 0.55	3.39 ± 0.61	3.98 ± 0.07	3.35 ± 0.05		3.80 ± 0.20	
	45-60	3.26 ± 0.25		3.41 ± 0.59	3.27 ± 0.06	3.58 ± 0.38	3.30 ± 0.05		3.63 ± 0.16	
	60-75	1.90 ± 0.10		3.31 ± 0.62	3.29 ± 0.60	2.99 ± 0.33	3.25 ± 0.07		2.74 ± 0.50	
	75-90	1.96 ± 0.12		2.85 ± 0.67	3.17 ± 0.60	1.97 ± 0.04	3.12 ± 0.14		3.08 ± 0.66	
	90-105			2.75 ± 0.00	2.36 ± 0.80	1.70 ± 0.10	2.79 ± 0.35		2.70 ± 0.71	
	TOTAL			23.32	22.24	21.08	20.93		22.76	
High	0-15	4.12 ± 0.24		3.86 ± 0.13	3.37 ± --	3.48 ± 0.16	2.49 ± 0.21		3.84 ± 0.16	
	15-30	3.64 ± 0.07		3.71 ± 0.25	3.28 ± --	3.56 ± 0.14	2.53 ± 0.19		4.01 ± 0.24	
	30-45	3.39 ± 0.45		3.92 ± 0.24	3.50 ± 0.28	3.78 ± 0.12	3.24 ± 0.12		3.94 ± 0.18	
	45-60	3.41 ± 0.70		3.47 ± 0.66	3.13 ± 0.52	4.00 ± 0.07	3.51 ± 0.08		3.92 ± 0.27	
	60-75	2.89 ± 0.69		2.73 ± 0.49	2.95 ± 0.50	2.92 ± 0.35	3.00 ± 0.35		3.15 ± 0.48	
	75-90	2.57 ± 0.50		2.74 ± 0.50	2.93 ± 0.33	2.95 ± 0.52	2.78 ± 0.31		2.72 ± 0.49	
	90-105			3.29 ± 0.00	2.44 ± 0.13	2.81 ± 0.35	2.23 ± 0.29		2.27 ± 0.50	
	TOTAL			23.71	21.60	23.49	19.78		23.86	

TABLE 11.10. CONTINUED

Treatment	Depth (cm)	8 July	14 July	22 July	29 July	5 August	13 August	19 August	26 August	6 September
Control	0-15	1.66 ± 0.25	1.85 ± 0.28	1.89 ± 0.29	0.87 ± 0.09	1.02 ± 0.18	0.56 ± 0.07	0.59 ± 0.23	0.63 ± 0.07	0.48 ± 0.08
	15-30	2.26 ± 0.15	1.71 ± 0.02	1.65 ± 0.12	1.47 ± 0.21	1.30 ± 0.02	0.88 ± 0.10	1.05 ± 0.19	1.29 ± 0.26	0.97 ± 0.09
	30-45	2.85 ± 0.62	2.71 ± 0.49	2.49 ± 0.25	1.65 ± 0.47	1.95 ± 0.27	1.52 ± 0.29	1.91 ± 0.10	1.73 ± 0.17	1.39 ± 0.18
	45-60	3.00 ± 0.65	2.61 ± 0.58	2.77 ± 0.50	1.64 ± 0.45	1.94 ± 0.52	1.77 ± 0.19	1.87 ± 0.31	1.99 ± 0.32	1.76 ± 0.24
	60-75	2.80 ± 0.49	2.50 ± 0.34	2.87 ± 0.42	2.00 ± 0.37	2.21 ± 0.09	1.77 ± 0.29	1.99 ± 0.20	1.85 ± 0.12	1.61 ± 0.25
	75-90	2.72 ± 0.44	2.42 ± 0.31	2.91 ± 0.28	1.94 ± 0.19	1.85 ± 0.18	2.11 ± 0.09	1.86 ± 0.08	1.72 ± 0.09	1.41 ± 0.08
	90-105	2.74 ± 0.59	2.44 ± 0.34	2.94 ± 0.30	2.13 ± 0.34	2.17 ± 0.23	1.89 ± 0.19	1.86 ± 0.22	2.24 ± 0.27	1.80 ± 0.26
	TOTAL	18.02	16.24	17.51	11.70	12.45	10.49	11.14	11.45	9.42
Low	0-15	1.43 ± 0.09		1.86 ± 0.07		1.07 ± 0.03		0.52 ± 0.05		0.49 ± 0.08
	15-30	2.51 ± 0.12		2.06 ± 0.06		1.21 ± 0.03		1.02 ± 0.14		1.07 ± 0.03
	30-45	3.24 ± 0.14		2.41 ± 0.24		1.62 ± 0.18		1.60 ± 0.20		1.44 ± 0.14
	45-60	3.65 ± 0.24		3.10 ± 0.16		2.33 ± 0.02		2.08 ± 0.01		1.88 ± 0.09
	60-75	2.98 ± 0.25		2.75 ± 0.14		2.31 ± 0.09		1.93 ± 0.04		1.63 ± 0.03
	75-90	2.71 ± 0.14		2.56 ± 0.17		2.30 ± 0.10		1.94 ± 0.21		1.57 ± 0.10
	90-105	2.45 ± 0.18		2.54 ± 0.14		2.36 ± 0.15		1.91 ± 0.09		1.71 ± 0.19
	TOTAL	18.97		17.27		13.21		11.01		9.78
Medium	0-15	1.60 ± 0.20		1.99 ± 0.27		1.18 ± 0.15		0.62 ± 0.14		0.60 ± 0.14
	15-30	2.40 ± 0.20		2.10 ± 0.13		1.53 ± 0.05		1.12 ± 0.15		1.03 ± 0.16
	30-45	3.15 ± 0.03		2.71 ± 0.16		1.86 ± 0.12		1.50 ± 0.01		1.43 ± 0.10
	45-60	3.27 ± 0.20		2.74 ± 0.19		1.87 ± 0.10		1.62 ± 0.02		1.58 ± 0.09
	60-75	3.42 ± 0.17		2.64 ± 0.31		2.05 ± 0.16		1.58 ± 0.11		1.46 ± 0.03
	75-90	2.97 ± 0.34		2.94 ± 0.16		1.97 ± 0.15		1.57 ± 0.14		1.59 ± 0.16
	90-105	2.32 ± 0.43		3.00 ± 0.36		2.05 ± 0.25		1.70 ± 0.20		1.69 ± 0.19
	TOTAL	19.12		18.13		12.50		9.71		9.37
High	0-15	1.77 ± 0.15		2.03 ± 0.06		2.17 ± 0.47		0.67 ± 0.07		0.61 ± 0.10
	15-30	2.61 ± 0.20		1.87 ± 0.16		1.96 ± 0.83		1.24 ± 0.15		1.11 ± 0.10
	30-45	3.37 ± 0.25		2.56 ± 0.16		2.36 ± 0.51		1.71 ± 0.05		1.55 ± 0.10
	45-60	3.65 ± 0.15		3.23 ± 0.33		2.75 ± 0.61		1.92 ± 0.08		1.76 ± 0.08
	60-75	3.08 ± 0.15		2.85 ± 0.58		2.18 ± 0.10		1.65 ± 0.05		1.38 ± 0.00
	75-90	2.71 ± 0.39		2.46 ± 0.30		2.16 ± 0.02		1.84 ± 0.12		1.53 ± 0.00
	90-105	2.58 ± 0.44		2.44 ± 0.38		3.14 ± 0.97		1.97 ± 0.26		1.63 ± 0.00
	TOTAL	19.77		17.45		16.71		11.00		9.57

[*] cm, $\bar{X}$ ± SE.

TABLE 11.11 SEASONAL DYNAMICS OF SOIL WATER, ZAPS I, 1977*

Treatment	Depth (cm)	14 April	20 April	29 April	5 May	12 May	17 May	23 May
Control	0-15	3.76 ± 0.19	3.31 ± 0.29	1.84 ± 0.19	1.69 ± 0.10	1.30 ± 0.03	2.18 ± 0.18	2.28 ± 0.16
	15-30	3.23 ± 0.13	3.30 ± 0.19	2.94 ± 0.31	2.99 ± 0.75	2.37 ± 0.36	1.96 ± 0.18	2.33 ± 0.41
	30-45	4.40 ± 0.18	3.93 ± 0.09	4.24 ± 0.17	3.61 ± 0.35	3.18 ± 0.23	3.38 ± 0.09	3.07 ± 0.18
	45-60	4.50 ± 0.25	4.44 ± 0.25	4.05 ± 0.25	3.95 ± 0.25	3.79 ± 0.30	3.52 ± 0.22	3.63 ± 0.35
	60-75	4.10 ± 0.21	3.75 ± 0.09	3.32 ± 0.29	3.80 ± 0.10	3.37 ± 0.31	3.60 ± 0.21	3.21 ± 0.25
	75-90	3.75 ± 0.28	2.43 ± 0.43	2.91 ± 0.37	3.33 ± 0.20	2.72 ± 0.50	2.93 ± 0.53	2.62 ± 0.21
	90-105	3.52 ± 0.42	2.28 ± 0.04	2.72 ± 0.47	3.06 ± 0.55	2.59 ± 0.38	2.85 ± 0.62	2.45 ± 0.21
	TOTAL	27.26	23.44	22.03	22.42	19.32	20.41	19.59
Low	0-15	3.78 ± 0.29		2.07 ± 0.16		0.99 ± 0.02		2.13 ± 0.12
	15-30	3.34 ± 0.05		2.91 ± 0.10		1.96 ± 0.05		2.10 ± 0.32
	30-45	3.78 ± 0.16		3.91 ± 0.05		3.27 ± 0.12		2.96 ± 0.14
	45-60	4.05 ± 0.01		4.17 ± 0.09		4.12 ± 0.10		3.91 ± 0.16
	60-75	4.08 ± 0.19		3.99 ± 0.07		3.69 ± 0.05		3.64 ± 0.08
	75-90	4.28 ± 0.16		3.47 ± 0.39		3.55 ± 0.17		3.54 ± 0.07
	90-105	3.82 ± 0.06		2.63 ± 0.89		3.59 ± 0.10		3.56 ± 0.06
	TOTAL	27.13		23.15		21.16		21.85
Medium	0-15	3.13 ± 0.11		1.84 ± 0.09		1.19 ± 0.18		2.55 ± 0.10
	15-30	3.46 ± 0.20		2.91 ± 0.04		1.89 ± 0.12		2.09 ± 0.17
	30-45	3.84 ± 0.36		3.97 ± 0.06		3.43 ± 0.04		3.20 ± 0.10
	45-60	4.19 ± 0.15		4.03 ± 0.08		3.67 ± 0.04		3.61 ± 0.14
	60-75	3.99 ± 0.05		3.71 ± 0.09		3.51 ± 0.09		3.52 ± 0.14
	75-90	3.91 ± 0.15		3.82 ± 0.26		3.71 ± 0.14		3.59 ± 0.19
	90-105	3.76 ± 0.13		3.45 ± 0.33		3.72 ± 0.17		3.52 ± 0.03
	TOTAL	26.27		23.73		21.11		22.07
High	0-15	3.49 ± 0.20		2.22 ± 0.18		1.25 ± 0.18		2.72 ± 0.10
	15-30	3.81 ± 0.15		3.33 ± 0.14		2.24 ± 0.19		2.34 ± 0.15
	30-45	3.98 ± 0.33		3.87 ± 0.04		3.43 ± 0.05		3.31 ± 0.09
	45-60	4.49 ± 0.28		4.27 ± 0.15		3.69 ± 0.13		3.91 ± 0.06
	60-75	4.04 ± 0.22		3.71 ± 0.12		3.70 ± 0.11		3.52 ± 0.10
	75-90	3.76 ± 0.44		3.76 ± 0.07		3.47 ± 0.12		3.45 ± 0.12
	90-105	3.76 ± 0.00		3.43 ± 0.19		2.91 ± 0.36		3.45 ± 0.06
	TOTAL	27.34		24.58		20.68		22.70

TABLE 11.11. CONTINUED

Treatment	Depth (cm)	30 May	16 June	2 July	15 July	2 August	18 August	9 September
Control	0-15	1.59 ± 0.05	3.64 ± 0.15	1.21 ± 0.13	0.93 ± 0.19	0.68 ± 0.12	0.72 ± 0.05	0.64 ± 0.06
	15-30	1.86 ± 0.22	3.29 ± 0.06	1.86 ± 0.10	1.39 ± 0.18	1.16 ± 0.08	1.10 ± 0.10	1.09 ± 0.05
	30-45	2.48 ± 0.12	3.08 ± 0.10	2.25 ± 0.18	1.98 ± 0.28	1.44 ± 0.09	1.82 ± 0.13	1.62 ± 0.16
	45-60	3.10 ± 0.24	2.97 ± 0.16	2.80 ± 0.23	2.46 ± 0.26	1.68 ± 0.31	2.09 ± 0.23	1.90 ± 0.36
	60-75	3.11 ± 0.26	2.50 ± 0.08	2.60 ± 0.40	2.58 ± 0.48	1.78 ± 0.25	2.29 ± 0.15	2.24 ± 0.00
	75-90	3.15 ± 0.30	2.30 ± 0.10	2.51 ± 0.07	2.42 ± 0.04	1.84 ± 0.05	2.37 ± 0.17	2.20 ± 0.00
	90-105	3.05 ± 0.36	2.28 ± 0.12	2.39 ± 0.30	2.63 ± 0.12	1.85 ± 0.15	2.38 ± 0.03	2.37 ± 0.00
	TOTAL	18.35	20.08	15.62	14.39	10.44	12.76	12.06
Low	0-15		3.13 ± 0.13			0.44 ± 0.02	0.68 ± 0.02	0.73 ± 0.03
	15-30		3.26 ± 0.11			0.83 ± 0.07	0.95 ± 0.10	1.33 ± 0.07
	30-45		3.10 ± 0.36			1.30 ± 0.10	1.37 ± 0.12	1.75 ± 0.13
	45-60		3.29 ± 0.30			1.76 ± 0.02	1.93 ± 0.12	1.90 ± 0.01
	60-75		3.05 ± 0.41			1.73 ± 0.04	1.61 ± 0.07	1.68 ± 0.11
	75-90		3.42 ± 0.51			1.82 ± 0.12	1.80 ± 0.17	1.76 ± 0.11
	90-105		3.16 ± 0.64			1.98 ± 0.05	2.08 ± 0.22	1.83 ± 0.16
	TOTAL		22.42			9.86	10.41	10.97
Medium	0-15		3.37 ± 0.19			0.57 ± 0.05	0.69 ± 0.13	0.80 ± 0.10
	15-30		3.60 ± 0.24			0.93 ± 0.11	1.21 ± 0.13	1.47 ± 0.02
	30-45		3.87 ± 0.23			1.47 ± 0.07	1.76 ± 0.15	1.87 ± 0.09
	45-60		2.75 ± 0.24			1.51 ± 0.11	1.71 ± 0.10	1.74 ± 0.08
	60-75		2.64 ± 0.17			1.52 ± 0.17	1.69 ± 0.10	1.67 ± 0.10
	75-90		2.92 ± 0.15			1.61 ± 0.23	1.91 ± 0.17	1.73 ± 0.16
	90-105		3.21 ± 0.17			1.80 ± 0.24	1.90 ± 0.17	1.82 ± 0.16
	TOTAL		22.36			9.42	10.87	11.09
High	0-15		3.57 ± 0.06			0.64 ± 0.12	0.62 ± 0.12	0.59 ± 0.17
	15-30		3.38 ± 0.31			1.03 ± 0.13	0.97 ± 0.13	1.33 ± 0.13
	30-45		3.34 ± 0.45			1.44 ± 0.07	1.38 ± 0.03	1.72 ± 0.04
	45-60		3.20 ± 0.12			1.96 ± 0.15	1.69 ± 0.05	1.87 ± 0.18
	60-75		3.14 ± 0.18			1.88 ± 0.28	1.76 ± 0.07	1.74 ± 0.10
	75-90		3.29 ± 0.24			1.97 ± 0.11	2.06 ± 0.17	2.23 ± 0.08
	90-105		3.09 ± 0.10			1.92 ± 0.10	1.81 ± 0.10	2.10 ± 0.26
	TOTAL		23.01			10.85	10.29	11.58

---

* cm, $\bar{X}$ ± SE.

TABLE 11.12. SEASONAL DYNAMICS OF SOIL WATER, ZAPS II, 1976*

Treatment	Depth (cm)	20 March	14 April	20 April
Control	0-15	3.64 ± 0.48	3.31 ± 0.12	3.67 ± 0.08
	15-30	2.95 ± 0.23	2.74 ± 0.02	3.63 ± 0.39
	30-45	2.22 ± 0.36	2.41 ± 0.55	2.64 ± 0.47
	45-60	1.97 ± 0.68	2.42 ± 0.54	2.48 ± 0.47
	60-75	1.93 ± 0.24	2.09 ± 0.48	2.11 ± 0.28
	75-90	1.60 ± 0.05	1.81 ± 0.12	1.91 ± 0.13
	90-105			
	TOTAL			
Low	0-15	4.35 ± 0.37	3.82 ± 0.37	
	15-30	3.08 ± 0.09	2.61 ± 0.96	
	30-45	2.89 ± 0.35	2.87 ± 0.95	
	45-60	2.75 ± 0.37	2.84 ± 0.68	
	60-75	2.23 ± 0.05	2.71 ± 0.68	
	75-90	2.41 ± 0.00	2.90 ± 0.00	
	90-105			
	TOTAL			
Medium	0-15	4.25 ± 0.41	3.34 ± 0.10	
	15-30	3.49 ± 0.17	3.23 ± 0.48	
	30-45	3.40 ± 0.51	3.39 ± 0.80	
	45-60	3.11 ± 0.81	3.22 ± 0.80	
	60-75	3.06 ± 0.00	3.00 ± 0.71	
	75-90	2.93 ± 0.00	2.47 ± 0.22	
	90-105		2.80 ± 0.00	
	TOTAL		21.45	
High	0-15	3.82 ± 0.11	3.47 ± 0.08	
	15-30	2.61 ± 0.09	2.85 ± 0.35	
	30-45	1.79 ± 0.04	2.30 ± 0.26	
	45-60	1.65 ± 0.05	1.86 ± 0.07	
	60-75	1.66 ± 0.04	1.85 ± 0.09	
	75-90	1.68 ± 0.00	1.58 ± 0.26	
	90-105		1.66 ± 0.00	
	TOTAL		15.57	

TABLE 11.12. CONTINUED

Treatment	Depth (cm)	5 May	16 May	26 May
Control	0-15	4.23 ± 0.15	3.59 ± 0.09	3.11 ± 0.29
	15-30	3.41 ± 0.10	2.92 ± 0.35	3.08 ± 0.05
	30-45	3.24 ± 0.29	3.40 ± 0.16	3.21 ± 0.12
	45-60	3.04 ± 0.74	2.94 ± 0.50	2.55 ± 0.36
	60-75	1.92 ± 0.56	2.15 ± 0.50	1.75 ± 0.07
	75-90	1.81 ± 0.48	1.97 ± 0.39	1.56 ± 0.11
	90-105		1.44 ± 0.38	1.68 ± 0.14
	TOTAL		18.39	16.93
Low	0-15	4.43 ± 0.12		2.87 ± 0.42
	15-30	4.34 ± 0.36		3.42 ± 0.33
	30-45	3.97 ± 0.21		3.44 ± 0.36
	45-60	2.90 ± 0.51		3.49 ± 0.65
	60-75	2.69 ± 0.54		2.64 ± 0.65
	75-90	2.17 ± 0.00		2.11 ± 0.26
	90-105			1.93 ± 0.13
	TOTAL			19.89
Medium	0-15	3.77 ± 0.20		2.55 ± 0.11
	15-30	3.91 ± 0.23		3.04 ± 0.41
	30-45	4.00 ± 0.19		3.48 ± 0.38
	45-60	13.48 ± 0.55		3.09 ± 0.57
	60-75	2.83 ± 0.85		2.87 ± 0.72
	75-90	2.99 ± 0.73		2.92 ± 0.63
	90-105			2.52 ± 0.66
	TOTAL			20.47
High	0-15	4.08 ± 0.07		2.73 ± 0.09
	15-30	3.83 ± 0.10		2.95 ± 0.17
	30-45	3.91 ± 0.11		3.29 ± 0.05
	45-60	3.64 ± 0.26		2.48 ± 0.50
	60-75	2.34 ± 0.22		1.86 ± 0.22
	75-90	1.66 ± 0.05		1.55 ± 0.02
	90-105			1.55 ± 0.06
	TOTAL			16.41

TABLE 11.12. CONTINUED

Treatment	Depth (cm)	2 June	13 June	21 June
Control	0-15	2.22 ± 0.17	2.65 ± 0.23	3.26 ± 0.21
	15-30	2.46 ± 0.16	1.86 ± 0.09	3.18 ± 0.36
	30-45	2.36 ± 0.16	2.05 ± 0.11	3.00 ± 0.14
	45-60	2.50 ± 0.26	1.94 ± 0.10	2.17 ± 0.17
	60-75	1.73 ± 0.19	1.48 ± 0.04	1.68 ± 0.04
	75-90	1.72 ± 0.03	1.40 ± 0.06	1.55 ± 0.06
	90-105	1.94 ± 0.06	1.55 ± 0.05	1.57 ± 0.09
	TOTAL	14.93	12.93	16.41
Low	0-15			3.64 ± 0.14
	15-30			4.16 ± 0.26
	30-45			3.35 ± 0.15
	45-60			2.73 ± 0.22
	60-75			2.49 ± 0.17
	75-90			2.16 ± 0.09
	90-105			2.26 ± 0.38
	TOTAL			20.81
Medium	0-15			3.38 ± 0.21
	15-30			3.91 ± 0.20
	30-45			3.84 ± 0.17
	45-60			3.08 ± 0.35
	60-75			2.74 ± 0.48
	75-90			2.90 ± 0.47
	90-105			2.75 ± 0.45
	TOTAL			22.61
High	0-15			3.18 ± 0.18
	15-30			3.37 ± 0.09
	30-45			3.35 ± 0.16
	45-60			2.40 ± 0.35
	60-75			1.77 ± 0.12
	75-90			1.64 ± 0.03
	90-105			1.64 ± 0.04
	TOTAL			[illegible]7.34

TABLE 11.12. CONTINUED

Treatment	Depth (cm)	24 June	1 July	8 July
Control	0-15	3.80 ± 0.16	2.69 ± 0.23	1.84 ± 0.10
	15-30	3.27 ± 0.24	3.11 ± 0.32	2.21 ± 0.23
	30-45	2.88 ± 0.20	2.70 ± 0.12	2.13 ± 0.19
	45-60	2.59 ± 0.15	2.44 ± 0.24	1.89 ± 0.14
	60-75	1.99 ± 0.05	1.85 ± 0.10	1.47 ± 0.07
	75-90	1.60 ± 0.10	1.59 ± 0.09	1.49 ± 0.04
	90-105	1.70 ± 0.09	1.63 ± 0.04	1.51 ± 0.14
	TOTAL	17.84	16.01	12.54
Low	0-15		3.01 ± 0.04	
	15-30		3.50 ± 0.03	
	30-45		2.78 ± 0.11	
	45-60		2.37 ± 0.22	
	60-75		2.22 ± 0.10	
	75-90		2.07 ± 0.03	
	90-105		2.11 ± 0.35	
	TOTAL		18.06	
Medium	0-15		2.58 ± 0.13	
	15-30		2.96 ± 0.01	
	30-45		2.83 ± 0.23	
	45-60		2.47 ± 0.28	
	60-75		2.50 ± 0.42	
	75-90		2.60 ± 0.35	
	90-105		2.39 ± 0.48	
	TOTAL		18.33	
High	0-15		2.80 ± 0.04	
	15-30		3.05 ± 0.17	
	30-45		2.72 ± 0.20	
	45-60		1.88 ± 0.15	
	60-75		1.63 ± 0.07	
	75-90		1.60 ± 0.05	
	90-105		1.65 ± 0.02	
	TOTAL		15.33	

TABLE 11.12. CONTINUED

Treatment	Depth (cm)	15 July	22 July	29 July
Control	0-15	2.50 ± 0.23	2.20 ± 0.37	1.43 ± 0.12
	15-30	2.03 ± 0.05	2.01 ± 0.23	1.59 ± 0.24
	30-45	1.93 ± 0.13	2.03 ± 0.22	1.63 ± 0.19
	45-60	2.05 ± 0.14	2.22 ± 0.05	1.85 ± 0.03
	60-75	1.79 ± 0.14	1.87 ± 0.14	1.62 ± 0.06
	75-90	1.66 ± 0.05	1.56 ± 0.02	1.44 ± 0.05
	90-105	1.65 ± 0.05	1.69 ± 0.11	1.45 ± 0.04
	TOTAL	13.61	13.57	11.02
Low	0-15	2.14 ± 0.24		1.46 ± 0.15
	15-30	2.29 ± 0.19		1.93 ± 0.10
	30-45	2.38 ± 0.09		2.08 ± 0.03
	45-60	2.27 ± 0.24		1.99 ± 0.16
	60-75	2.21 ± 0.01		1.84 ± 0.09
	75-90	2.03 ± 0.05		1.89 ± 0.13
	90-105	2.04 ± 0.21		2.01 ± 0.29
	TOTAL	15.36		13.20
Medium	0-15	2.36 ± 0.24		1.31 ± 0.16
	15-30	2.34 ± 0.13		1.48 ± 0.20
	30-45	2.52 ± 0.12		1.98 ± 0.16
	45-60	2.47 ± 0.13		1.97 ± 0.13
	60-75	2.52 ± 0.10		2.02 ± 0.22
	75-90	2.56 ± 0.14		2.35 ± 0.30
	90-105	2.38 ± 0.20		2.22 ± 0.30
	TOTAL	17.16		13.34
High	0-15	2.34 ± 0.07		1.22 ± 0.03
	15-30	1.95 ± 0.04		1.46 ± 0.11
	30-45	2.15 ± 0.12		1.75 ± 0.02
	45-60	1.91 ± 0.13		1.64 ± 0.15
	60-75	1.64 ± 0.13		1.59 ± 0.06
	75-90	1.64 ± 0.02		1.48 ± 0.08
	90-105	1.72 ± 0.08		1.56 ± 0.05
	TOTAL	13.35		10.69

TABLE 11.12. CONTINUED

Treatment	Depth (cm)	5 August	13 August	19 August
Control	0-15	2.02 ± 0.19	1.28 ± 0.13	1.05 ± 0.07
	15-30	1.23 ± 0.26	1.60 ± 0.14	1.45 ± 0.15
	30-45	1.65 ± 0.10	1.64 ± 0.13	1.50 ± 0.11
	45-60	1.76 ± 0.05	1.84 ± 0.09	1.52 ± 0.08
	60-75	1.63 ± 0.08	1.69 ± 0.05	1.49 ± 0.02
	75-90	1.28 ± 0.26	1.62 ± 0.03	1.41 ± 0.05
	90-105	1.48 ± 0.12	1.68 ± 0.20	1.51 ± 0.20
	TOTAL	11.06	11.33	9.93
Low	0-15		1.18 ± 0.09	
	15-30		1.86 ± 0.13	
	30-45		1.94 ± 0.16	
	45-60			
	60-75			
	75-90			
	90-105			
	TOTAL			
Medium	0-15		1.10 ± 0.13	
	15-30		1.48 ± 0.19	
	30-45		1.94 ± 0.15	
	45-60			
	60-75			
	75-90			
	90-105			
	TOTAL			
High	0-15		1.06 ± 0.06	
	15-30		1.37 ± 0.12	
	30-45		1.50 ± 0.09	
	45-60			
	60-75			
	75-90			
	90-105			
	TOTAL			

TABLE 11.12. CONTINUED

Treatment	Depth (cm)	26 August	3 September	10 September
Control	0-15	1.25 ± 0.09	1.01 ± 0.05	1.21 ± 0.09
	15-30	1.58 ± 0.18	1.23 ± 0.09	1.42 ± 0.22
	30-45	1.54 ± 0.13	1.32 ± 0.21	1.44 ± 0.09
	45-60	1.80 ± 0.03	1.46 ± 0.07	1.64 ± 0.05
	60-75	1.61 ± 0.10	1.42 ± 0.01	1.44 ± 0.06
	75-90	1.61 ± 0.04	1.26 ± 0.06	1.39 ± 0.04
	90-105	1.60 ± 0.10	1.28 ± 0.14	1.47 ± 0.09
	TOTAL	11.00	8.97	10.02
Low	0-15	1.39 ± 0.12		1.20 ± 0.07
	15-30	1.98 ± 0.13		1.93 ± 0.08
	30-45	2.05 ± 0.06		2.11 ± 0.06
	45-60	2.26 ± 0.21		2.07 ± 0.19
	60-75	2.03 ± 0.22		1.82 ± 0.07
	75-90	1.99 ± 0.14		1.95 ± 0.05
	90-105	2.12 ± 0.20		2.01 ± 0.18
	TOTAL	13.82		13.08
Medium	0-15	1.22 ± 0.12		1.17 ± 0.19
	15-30	1.67 ± 0.12		1.67 ± 0.27
	30-45	1.91 ± 0.09		1.95 ± 0.15
	45-60	1.95 ± 0.12		1.90 ± 0.16
	60-75	2.02 ± 0.17		2.00 ± 0.22
	75-90	2.06 ± 0.04		2.12 ± 0.15
	90-105	1.88 ± 0.06		2.14 ± 0.21
	TOTAL	12.71		12.95
High	0-15	1.11 ± 0.08		1.05 ± 0.08
	15-30	1.55 ± 0.18		1.25 ± 0.15
	30-45	1.70 ± 0.06		1.59 ± 0.08
	45-60	1.51 ± 0.05		1.57 ± 0.09
	60-75	1.62 ± 0.04		1.43 ± 0.02
	75-90	1.63 ± 0.05		1.37 ± 0.05
	90-105	1.72 ± 0.15		1.48 ± 0.10
	TOTAL	10.83		9.72

[*] cm, $\bar{X}$ ± SE.

TABLE 11.13. SEASONAL DYNAMICS OF SOIL WATER, ZAPS II, 1977*

Treatment	Depth (cm)	20 April	29 April	5 May	12 May	17 May	23 May	30 May	16 June	15 July	16 August	9 September
Control	0-15	3.27 ± 0.09	2.27 ± 0.08	2.08 ± 0.07	1.70 ± 0.05	2.80 ± 0.05	2.77 ± 0.11	2.13 ± 0.07	4.16 ± 0.07	1.02 ± 0.28	1.17 ± 0.08	1.12 ± 0.10
	15-30	3.56 ± 0.33	3.20 ± 0.15	2.82 ± 0.29	2.51 ± 0.34	2.66 ± 0.26	2.33 ± 0.22	2.16 ± 0.18	3.36 ± 0.29	1.49 ± 0.14	1.60 ± 0.20	1.48 ± 0.16
	30-45	3.78 ± 0.07	3.71 ± 0.17	3.35 ± 0.04	2.93 ± 0.10	3.10 ± 0.10	2.62 ± 0.06	2.44 ± 0.05	2.28 ± 0.22	1.70 ± 0.23	1.63 ± 0.14	1.11 ± 0.19
	45-60	3.87 ± 0.10	3.63 ± 0.03	3.65 ± 0.17	3.28 ± 0.16	3.40 ± 0.10	3.03 ± 0.17	2.95 ± 0.25	2.22 ± 0.03	1.94 ± 0.08	1.66 ± 0.05	1.60 ± 0.13
	60-75	3.49 ± 0.57	3.35 ± 0.35	3.72 ± 0.06	3.35 ± 0.21	3.22 ± 0.25	3.08 ± 0.27	2.65 ± 0.22	2.29 ± 0.13	1.62 ± 0.01	1.70 ± 0.05	1.55 ± 0.08
	75-90	3.15 ± 0.47	2.90 ± 0.55	3.02 ± 0.42	2.84 ± 0.56	2.44 ± 0.42	2.59 ± 0.68	2.46 ± 0.40	2.10 ± 0.08	1.66 ± 0.03	1.56 ± 0.05	1.57 ± 0.05
	90-105	2.97 ± 0.66	2.49 ± 0.64	2.57 ± 0.68	2.48 ± 0.64	2.76 ± 0.77	2.32 ± 0.65	2.22 ± 0.32	2.02 ± 0.28	1.56 ± 0.08	1.65 ± 0.03	1.40 ± 0.08
	TOTAL	24.09	21.56	21.22	19.09	20.37	18.74	17.01	18.43	10.99	10.98	9.82
Low	0-15	3.51 ± 0.06		2.30 ± 0.12		2.95 ± 0.03		2.44 ± 0.08		1.50 ± 0.03	1.25 ± 0.01	1.27 ± 0.03
	15-30	4.39 ± 0.17		3.40 ± 0.07		2.78 ± 0.17		2.63 ± 0.15		2.05 ± 0.15	1.97 ± 0.13	1.82 ± 0.18
	30-45	4.68 ± 0.17		4.02 ± 0.08		4.03 ± 0.77		2.92 ± 0.06		2.27 ± 0.06	2.18 ± 0.05	1.94 ± 0.10
	45-60	4.72 ± 0.34		4.23 ± 0.21		3.91 ± 0.25		3.28 ± 0.02		2.24 ± 0.07	2.19 ± 0.18	1.95 ± 0.17
	60-75	4.56 ± 0.36		4.30 ± 0.10		4.17 ± 0.02		3.68 ± 0.07		2.24 ± 0.03	2.04 ± 0.06	1.85 ± 0.09
	75-90	4.50 ± 0.07		4.09 ± 0.16		4.03 ± 0.20		3.60 ± 0.27		2.31 ± 0.23	2.00 ± 0.09	1.83 ± 0.08
	90-105	3.89 ± 0.64		3.85 ± 0.43		4.18 ± 0.74		3.69 ± 0.37		2.69 ± 0.50	2.12 ± 0.28	1.98 ± 0.17
	TOTAL	30.25		26.19		26.06		22.24		15.30	13.75	12.64
Medium	0-15	2.82 ± 0.25		2.16 ± 0.19		2.76 ± 0.19		2.11 ± 0.02		1.34 ± 0.08	1.17 ± 0.16	1.15 ± 0.15
	15-30	3.55 ± 0.26		3.04 ± 0.15		2.50 ± 0.18		2.29 ± 0.18		1.81 ± 0.24	1.68 ± 0.27	1.71 ± 0.27
	30-45	4.10 ± 0.19		3.98 ± 0.12		3.32 ± 0.12		2.64 ± 0.13		2.14 ± 0.10	2.01 ± 0.15	1.94 ± 0.08
	45-60	4.37 ± 0.17		3.96 ± 0.06		3.57 ± 0.27		3.26 ± 0.02		2.23 ± 0.17	2.04 ± 0.15	1.89 ± 0.15
	60-75	4.05 ± 0.60		4.08 ± 0.13		3.49 ± 0.63		3.42 ± 0.21		2.20 ± 0.15	2.11 ± 0.13	2.47 ± 0.50
	75-90	3.67 ± 0.67		4.27 ± 0.08		3.63 ± 0.52		3.62 ± 0.09		2.51 ± 0.19	2.14 ± 0.07	2.05 ± 0.06
	90-105	3.61 ± 0.67		3.80 ± 0.32		3.41 ± 0.61		3.26 ± 0.46		3.01 ± 0.69	2.05 ± 0.08	1.82 ± 0.18
	TOTAL	26.27		25.29		22.67		20.60		15.25	13.21	13.04
High	0-15	2.82 ± 0.12		2.03 ± 0.13		2.73 ± 0.04		2.33 ± 0.12		1.50 ± 0.22	1.27 ± 0.12	1.08 ± 0.06
	15-30	3.27 ± 0.24		2.78 ± 0.26		2.44 ± 0.15		1.99 ± 0.11		1.76 ± 0.06	1.42 ± 0.15	1.38 ± 0.13
	30-45	3.61 ± 0.33		3.55 ± 0.17		3.21 ± 0.27		2.63 ± 0.14		1.99 ± 0.15	1.78 ± 0.12	1.58 ± 0.06
	45-60	3.78 ± 0.07		3.75 ± 0.14		3.33 ± 0.17		3.16 ± 0.12		1.98 ± 0.04	1.67 ± 0.07	1.59 ± 0.07
	60-75	3.81 ± 0.11		3.72 ± 0.14		3.47 ± 0.09		3.20 ± 0.05		1.89 ± 0.09	1.68 ± 0.07	1.63 ± 0.08
	75-90	3.46 ± 0.22		3.69 ± 0.12		3.54 ± 0.05		3.51 ± 0.16		1.89 ± 0.12	1.64 ± 0.10	1.65 ± 0.07
	90-105	3.11 ± 0.57		3.55 ± 0.16		3.44 ± 0.26		3.39 ± 0.11		1.94 ± 0.11	1.66 ± 0.06	1.54 ± 0.21
	TOTAL	23.86		23.07		22.16		20.21		12.95	11.13	10.45

* cm, $\bar{X}$ ± SE.

TABLE 11.14. GROWING SEASON PRECIPITATION (MM) FOR THE ZAPS SITES FOR 1975, 1976, AND 1977 COMPARED WITH LONG-TERM AVERAGE AT BROADUS, MONTANA

Date	ZAPS I (mm) 1975	ZAPS I (mm) 1976	ZAPS I (mm) 1977	ZAPS II (mm) 1976	ZAPS II (mm) 1977	Long-term average*
May						
1-15	72	29	17	22	18	
16-31	32	68	28	22	28	
Total	104	97	45	44	46	57
June						
1-15	34	67	77	57	79	
16-31	75	35	14	37	19	
Total	109	102	91	94	98	80
July						
1-15	12	25	10	27	7	
16-31	17	13	8	14	7	
Total	29	38	18	41	14	35
August						
1-15	12	4	13	10	11	
16-31	2	6	5	7	7	
Total	14	10	18	17	18	27
September						
1-15	2	5	2	5	2	
16-31	4	9	34	9	38	
Total	6	14	36	14	40	30

* Long-term average as recorded at Broadus, Montana located on Powder River approximately 50 km northeast of sites, 1932-1977.

TABLE 11.15. METEOROLOGICAL VARIABLES AT ZAPS I, 1976

Air temperature (°C)	4/11-17	18-24	4/25-5/1	Apr	5/2-8	9-15	16-22	23-29	May	5/30-6/5	6-12	13-9	20-26	6/27-7/3	June
Average	13	7	5	7	11	14	15	13	13	18	19	12	14	19	15
Maximum	27	17	19	27	23	26	26	26	26	28	31	25	29	30	31
Minimum	5	-1	0	-1	-3	3	5	3	-3	8	8	5	5	8	5
Relative Humidity (%)															
Average	43	57	74	65	51	55	60	62	56	61	59	67	62	55	59
Maximum	79	99	99	99	99	99	99	99	99	99	99	99	99	99	99
Minimum	22	6	23	6	15	28	24	25	15	22	24	23	26	23	23

Air temperature (°C)	7/4-10	11-17	18-24	25-31	July	8/1-7	8-14	15-21	22-28	Aug	8/29-9/4	9/5-11	12-18	19-25	26-10/2
Average	24	22	24	23	22	22	23	25	21	21	24	20	20	17	17
Maximum	33	35	37	32	37	36	32	35	36	36	37	39	33	27	31
Minimum	15	10	15	13	10	13	13	16	3	3	13	4	10	10	2
Relative Humidity (%)															
Average	56	52	50	48	52	56	47	43	40	46	33	33	56	52	45
Maximum	94	99	99	91	99	99	97	99	99	99	65	99	99	99	99
Minimum	23	16	16	18	16	11	26	15	18	11	13	9	19	18	14

Air temperature (°C)	Sep	10/3-9	10-16	17-23	Oct
Average	18	10	--	--	15
Maximum	39	19	--	--	31
Minimum	2	5	--	--	5
Relative Humidity (%)					
Average	44	70	--	--	48
Maximum	99	99	--	--	99
Minimum	9	40	--	--	15

TABLE 11.16. METEOROLOGICAL VARIABLES AT ZAPS II, 1976

Air temperature (°C)	4/11-17	18-24	4/25-5/1	Apr	5/2-8	9-15	16-22	23-29	May	5/30-6/5	6-12	13-9	20-26	6/27-7/3	June
Average	10	6	5	6	10	13	15	14	12	19	20	13	15	19	19
Maximum	57	16	19	52	22	25	25	28	28	29	32	25	31	31	32
Minimum	51	0	0	-1	-3	3	5	4	-3	-6	8	5	6	9	-6
Relative Humidity (%)															
Average	--	63	74	71	53	55	62	59	56	60	60	68	69	63	62
Maximum	--	99	99	99	99	99	99	99	99	97	99	99	99	99	99
Minimum	--	30	26	30	22	31	30	22	22	24	22	14	30	31	14

Air temperature (°C)	7/4-10	11-17	18-24	25-31	July	8/1-7	8-14	15-21	22-28	Aug	8/29-9/4	9/5-11	12-18	19-25	26-10/2
Average	25	22	25	22	22	21	22	25	23	21	23	19	19	15	17
Maximum	33	35	37	32	37	36	31	35	35	36	35	38	32	27	31
Minimum	15	11	15	12	11	12	13	15	11	11	11	5	8	7	2
Relative Humidity (%)															
Average	64	56	54	52	57	67	51	46	46	50	36	40	60	59	47
Maximum	99	99	99	96	99	99	95	99	99	99	62	99	99	99	99
Minimum	34	21	22	23	21	19	29	21	24	19	17	7	25	22	19

Air temperature (°C)	Sep	10/3-9	10-16	17-23	Oct
Average	17	10	--	4	8
Maximum	38	20	--	11	31
Minimum	2	5	--	-3	-3
Relative Humidity (%)					
Average	50	72	--	60	57
Maximum	99	99	--	99	99
Minimum	7	43	--	38	20

TABLE 11.17. METEOROLOGICAL VARIABLES AT ZAPS I, 1977

Air temperature (°C)	4/14-23	24-30	Apr	5/1-7	8-14	15-21	22-28	May	5/29-6/4	6/5-11	12-18	19-25	26-7/2	June	7/3-9
Average	9	16	11	12	18	10	15	13	18	20	16	21	20	18	19
Maximum	21	26	26	23	26	20	27	27	31	30	24	33	30	33	30
Minimum	-17	6	-17	0	6	3	4	0	3	6	8	12	7	6	10
Relative Humidity (%)															
Average	50	38	43	48	49	65	56	53	46	56	67	52	43	53	54
Maximum	98	88	98	97	94	98	99	99	97	99	99	88	90	99	92
Minimum	18	19	18	19	16	22	20	16	25	30	31	28	22	22	23

Air temperature (°C)	10-16	17-23	24-30	July	7/31-8/6	8/7-13	14-20	21-27	28-9/13	Aug	9/4-10	11-17	18-24	25-10/1	Sep
Average	21	24	21	20	18	16	20	19	17	17	21	17	13	11	16
Maximum	35	38	35	38	28	30	31	35	33	35	33	31	25	22	33
Minimum	9	13	12	9	8	6	7	7	7	6	5	6	5	2	5
Relative Humidity (%)															
Average	49	43	53	49	58	59	53	65	58	58	49	55	64	74	58
Maximum	99	90	99	99	99	99	99	99	99	99	99	99	99	99	99
Minimum	14	12	19	12	25	15	25	14	19	14	18	19	24	29	18

Air temperature (°C)	10/2-8	9-15	Oct
Average	7	3	4
Maximum	17	15	17
Minimum	-1	-5	-5
Relative Humidity (%)			
Average	72	55	66
Maximum	99	99	99
Minimum	26	23	23

TABLE 11.18. METEOROLOGICAL VARIABLES AT ZAPS II, 1977

Air temperature (°C)	4/17-23	24-30	Apr	5/1-7	8-14	15-21	22-28	May	5/29-6/4	6/5-11	12-18	19-25	26-7/2	June	7/3-9
Average	8	16	11	13	19	10	15	13	18	20	16	20	21	18	19
Maximum	21	27	27	23	26	20	27	27	31	32	25	32	31	32	31
Minimum	0	5	0	1	7	3	4	1	4	7	8	10	6	6	9
Relative Humidity (%)															
Average	54	37	46	48	49	63	57	53	46	59	73	59	52	58	62
Maximum	98	89	98	94	92	94	96	96	96	96	97	97	96	97	98
Minimum	17	16	16	15	12	20	17	12	22	29	36	31	25	22	21

Air temperature (°C)	10-16	17-23	24-30	July	7/31-8/6	8/7-13	14-20	21-27	28-9/3	Aug	9/4-10	11-17	18-24	25-10/1	Sep
Average	21	25	23	21	20	18	20	19	17	18	20	17	13	11	15
Maximum	35	38	36	38	31	32	30	35	32	35	32	31	24	22	32
Minimum	8	15	12	8	10	8	8	7	6	6	4	6	5	2	4
Relative Humidity (%)															
Average	51	47	52	52	55	60	54	66	60	59	51	57	65	71	59
Maximum	97	96	98	98	96	98	96	98	98	98	96	96	96	96	96
Minimum	12	10	15	10	23	14	25	16	18	14	18	17	27	29	17

Air temperature (°C)	10/2-8	9-15	Oct
Average	6	3	4
Maximum	16	15	16
Minimum	-2	-4	-4
Relative Humidity (%)			
Average	67	52	61
Maximum	94	96	96
Minimum	24	21	21

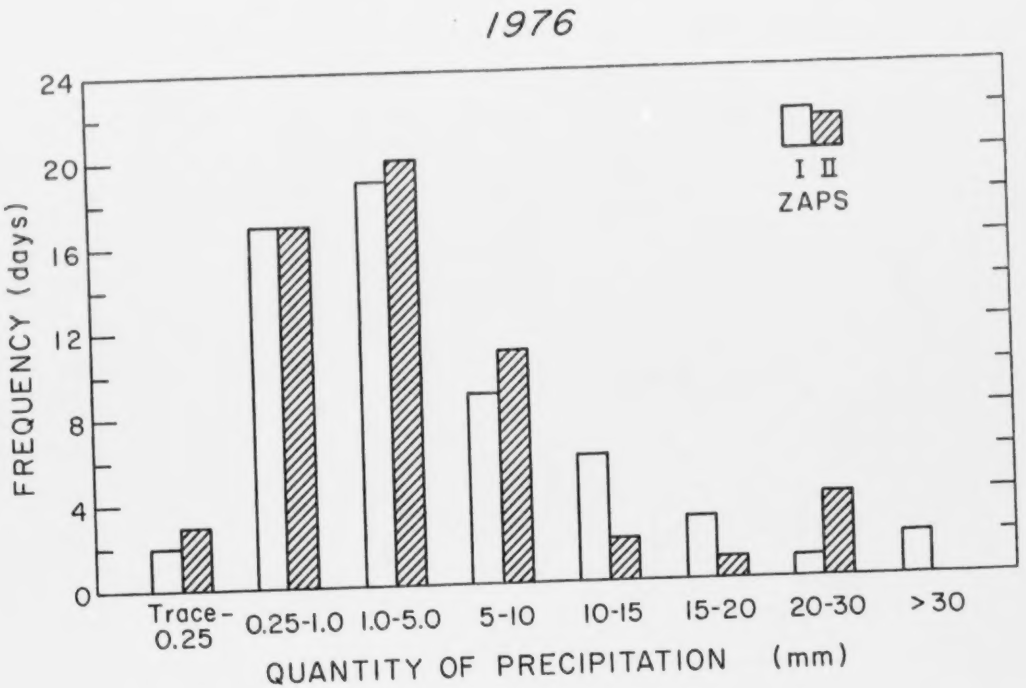


Figure 11.3. Histogram of incidence and quantity of precipitation, 1976.

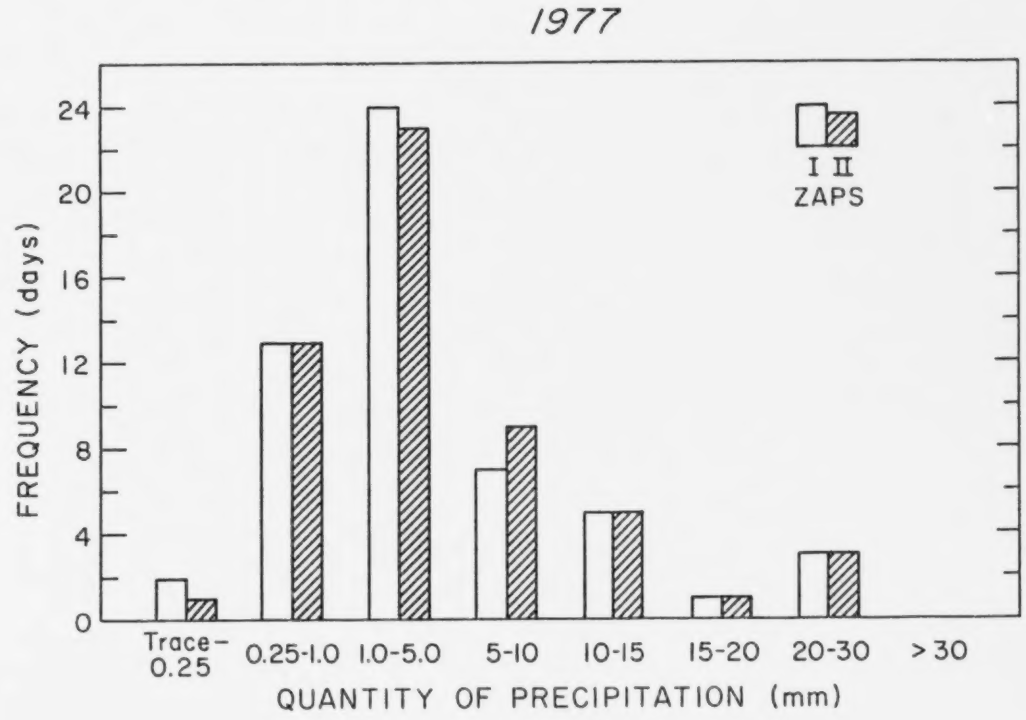


Figure 11.4. Histogram of incidence and quantity of precipitation, 1977.

METEOROLOGICAL VARIABLES AT ZAPS

Instrumentation

Air temperature and relative humidity were measured with a hydrothermograph (Bendix Model 594) placed in an instrument shelter 1.5 m above ground. Wind speed and direction were monitored at 2.1 m above ground with a portable system consisting of a balsa vane and 3-cup anemometer (Ecowind IIIB, Wong Laboratories). The threshold for both indicators was 33.5 cm/sec. Wind direction was recorded as an analog signal on a scale marked N-E-S-W-N, while wind speed was recorded as an integrated signal, a mark for every 0.8 km of air passage. The unit was DC powered and recorded on a Gulton recorder (Model 288/F2146).

Meteorological Variables

Data for air temperature (°C) and relative humidity (%) are presented in Tables 11.15 and 11.16 for ZAPS I and Tables 11.17 and 11.18 for ZAPS II for years 1976 and 1977, respectively. These tables summarize data by week and month, the monthly summary following the 7-day data.

Air Temperature

Monthly averages for ZAPS I and ZAPS II in 1976 and 1977 were similar, differing by no more than 1°C. However, air temperature was higher in 1977 on both ZAPS in April and June but lower in other months. The absence of freezing temperatures occurred in early May 1977 but not until after the first week of June in 1976. The warmest month in both years was July, which averaged 20°C or above in both years. Also, the average maximum was 37 to 38°C for July.

Relative Humidity

This undergoes characteristic diurnal fluctuations because warm air can hold more moisture. Most maximum RHs were 99% (saturated), which occurred at night. During the growing season at ZAPS I, June showed the highest of the minimum RHs, correlating with abundant precipitation in 1976 (Table 11.14). In 1977, June was the wettest month; hence, the minimum RHs were 22-31%. The lowest RH recorded for any month occurred in June 1977 at ZAPS II. July was also the month of least precipitation in 1977 (see Table 11.14).

Wind Speed and Direction

A series of histograms depicting wind speed in meters sec^{-1} and wind direction at eight points of the compass are in Figures 11.5 to 11.32. Each figure contains information for one month and shows the duration of a particular range of wind speeds in a given direction. The breakdown of figures is:

Figures 11.5 - 11.11: ZAPS I, 1976
11.12 - 11.18: ZAPS I, 1977
11.19 - 11.25: ZAPS II, 1976
11.26 - 11.32: ZAPS II, 1977

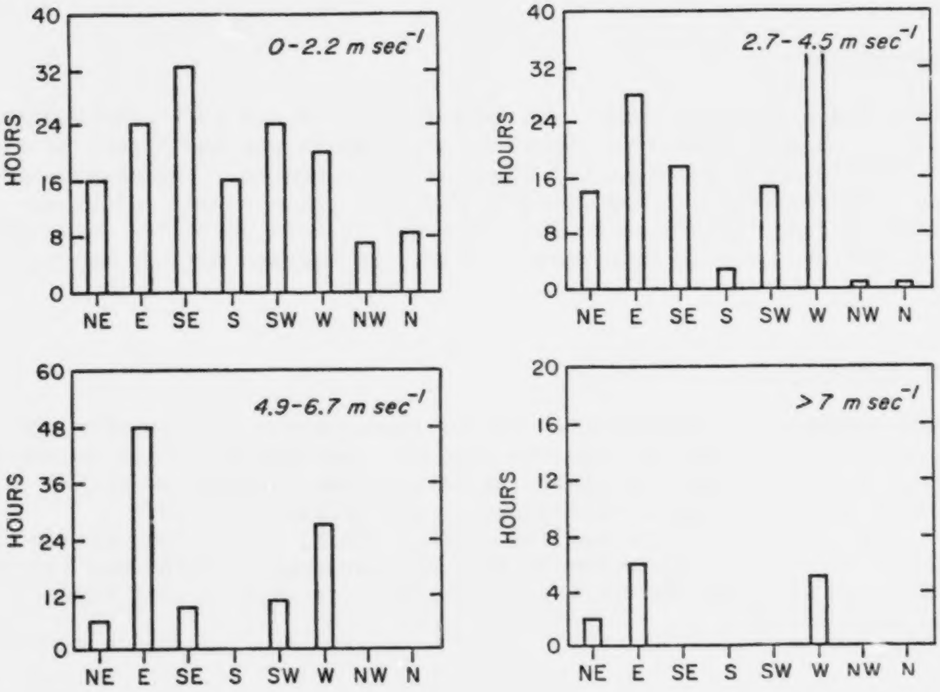


Figure 11.5. Histograms of wind speed and direction for ZAPS I, April, 1976.

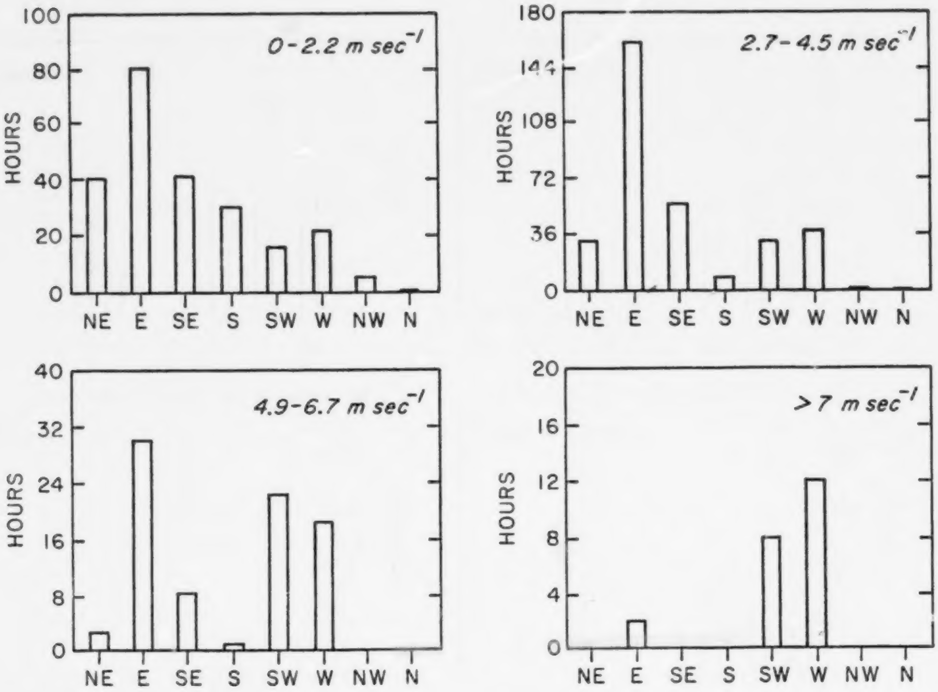


Figure 11.6. Histograms of wind speed and direction for ZAPS I, May, 1976.

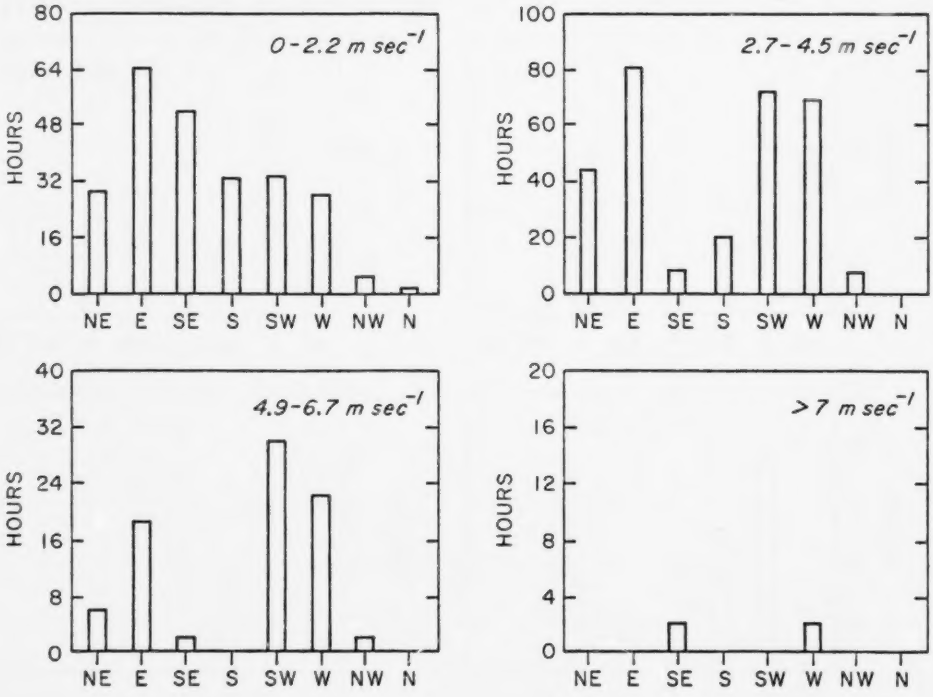


Figure 11.7. Histograms of wind speed and direction for ZAPS I, June, 1976.

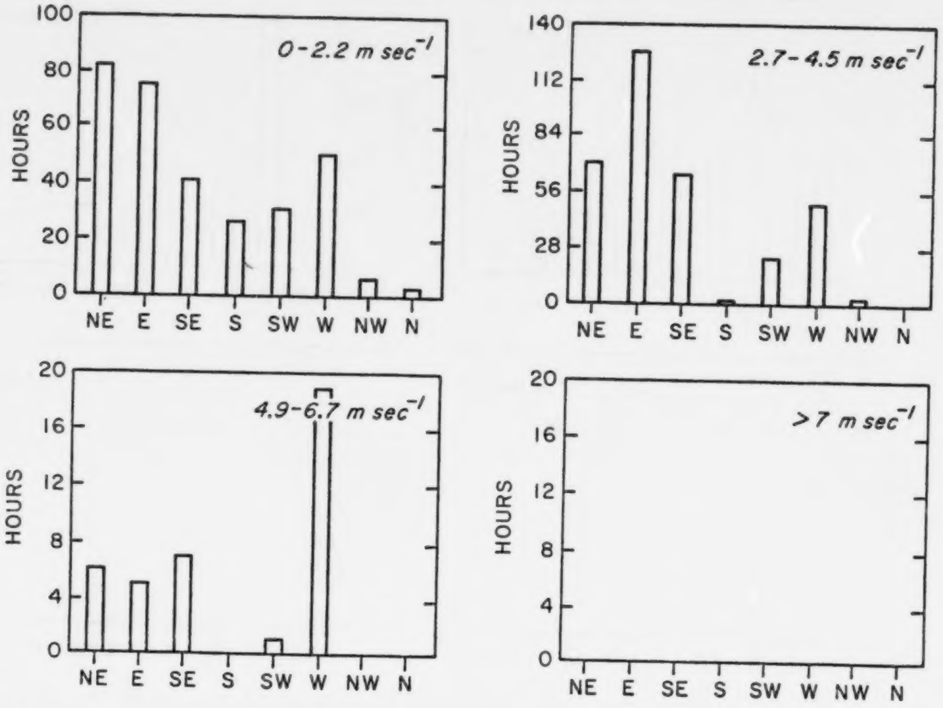


Figure 11.8. Histograms of wind speed and direction for ZAPS I, July, 1976.

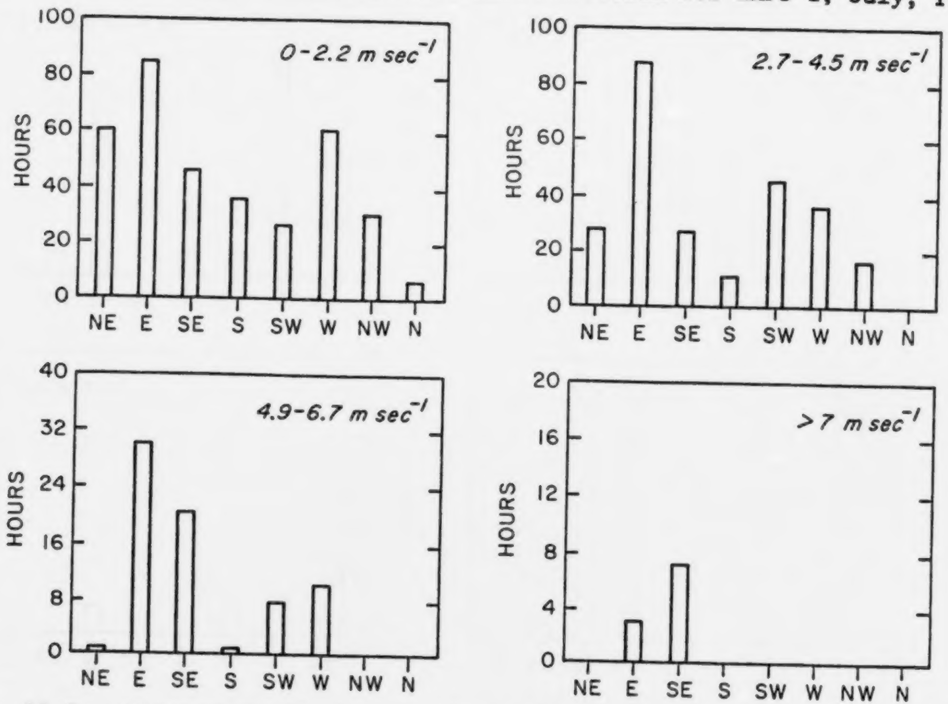


Figure 11.9. Histograms of wind speed and direction for ZAPS I, August, 1976.

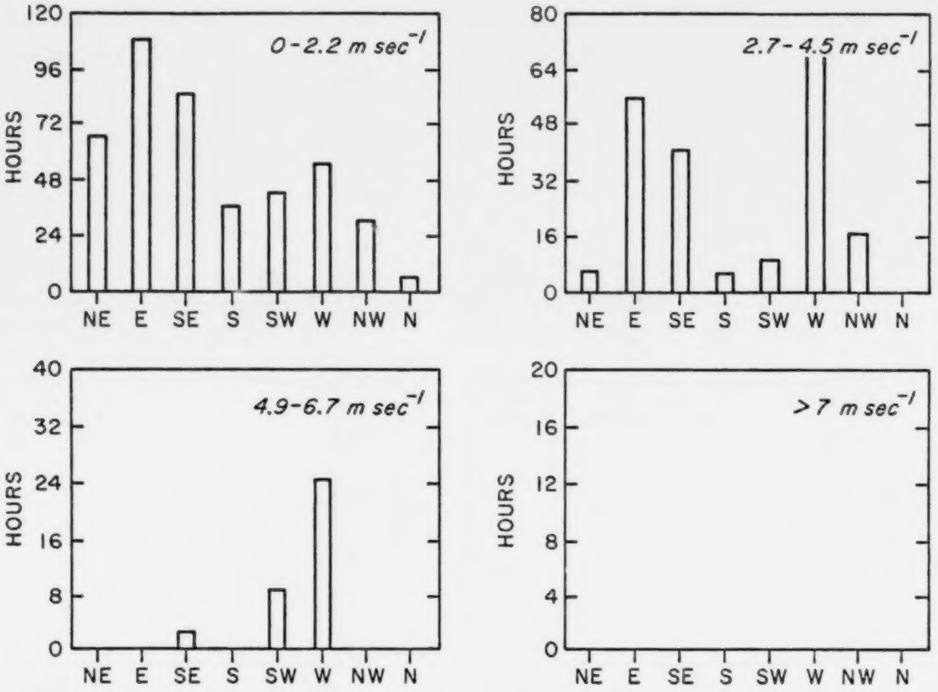


Figure 11.10. Histograms of wind speed and direction for ZAPS I, September, 1976.

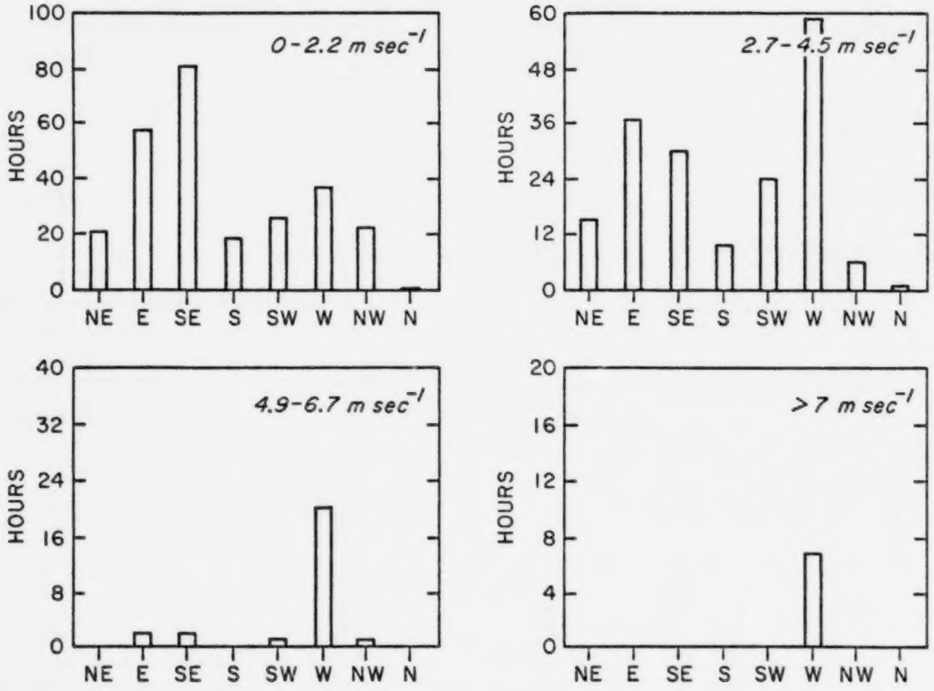


Figure 11.11. Histograms of wind speed and direction for ZAPS I, October, 1976.

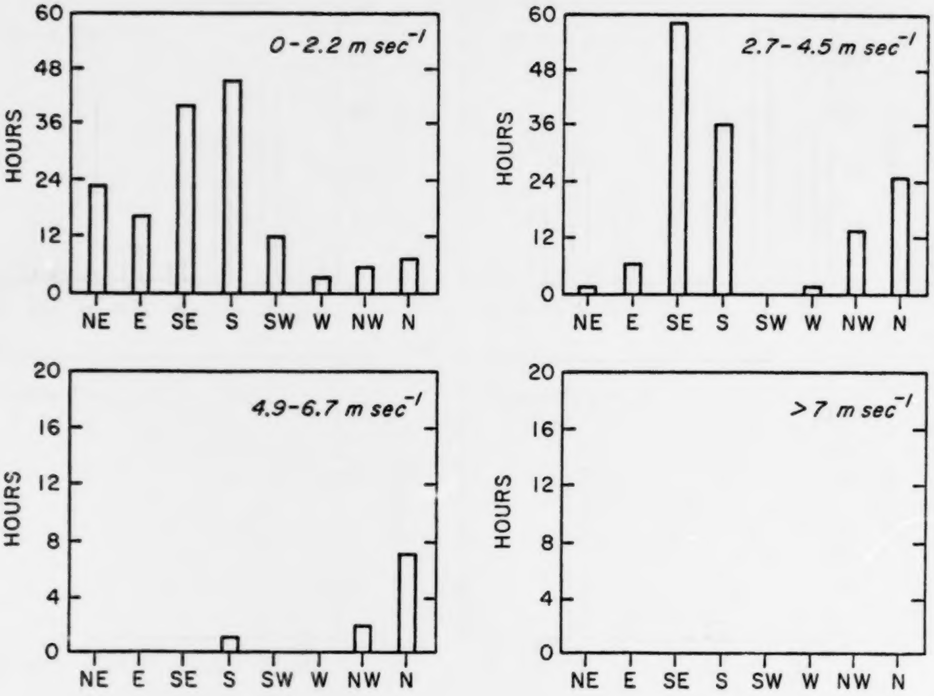


Figure 11.12. Histograms of wind speed and direction for ZAPS I, April, 1977.

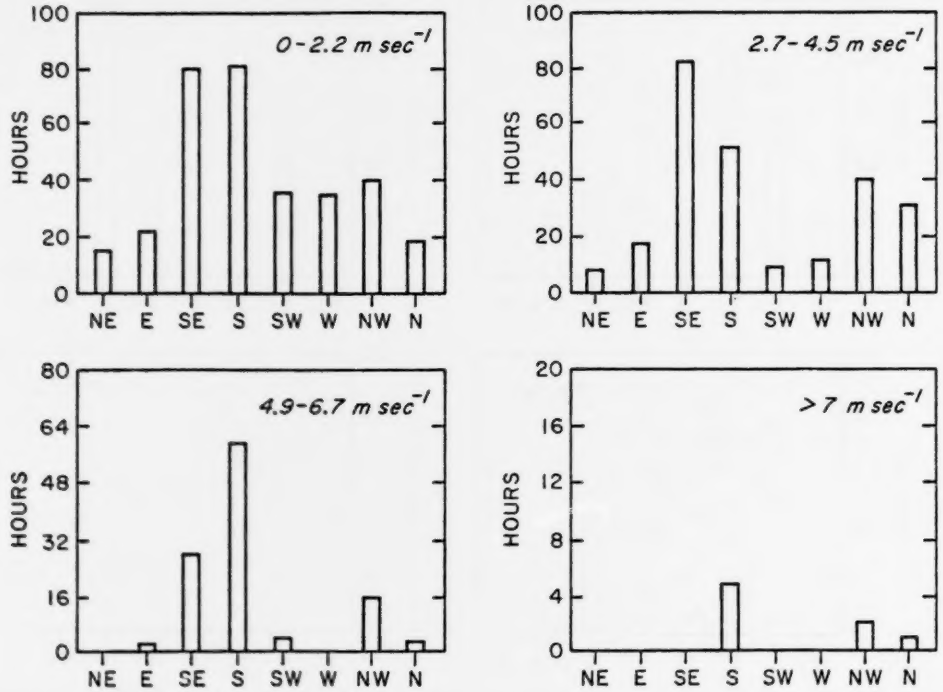


Figure 11.13. Histograms of wind speed and direction for ZAPS I, May, 1977.

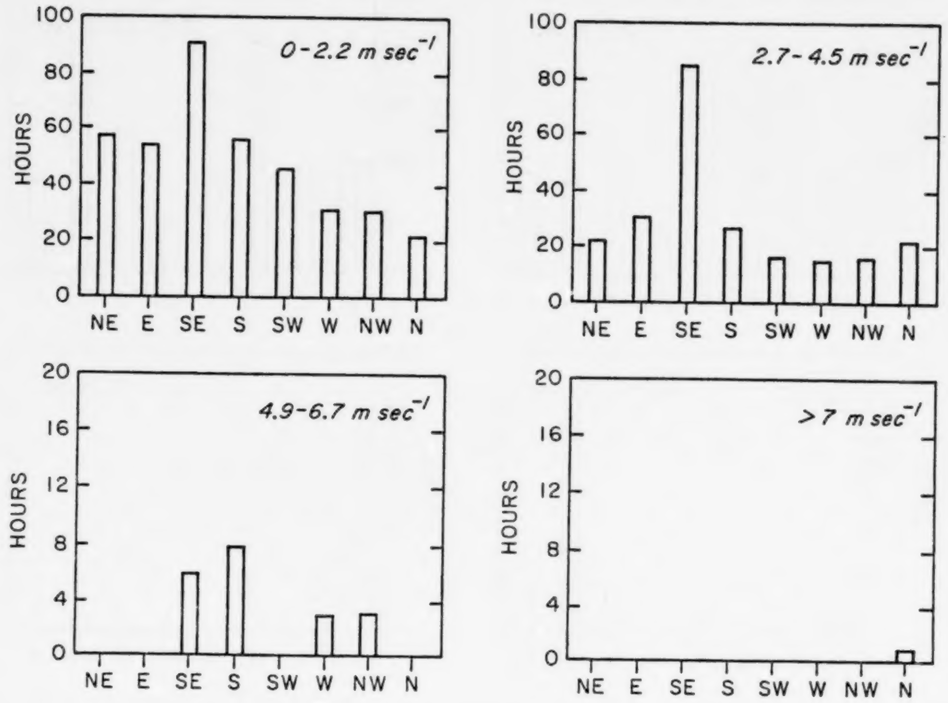


Figure 11.14. Histograms of wind speed and direction for ZAPS I, June, 1977.

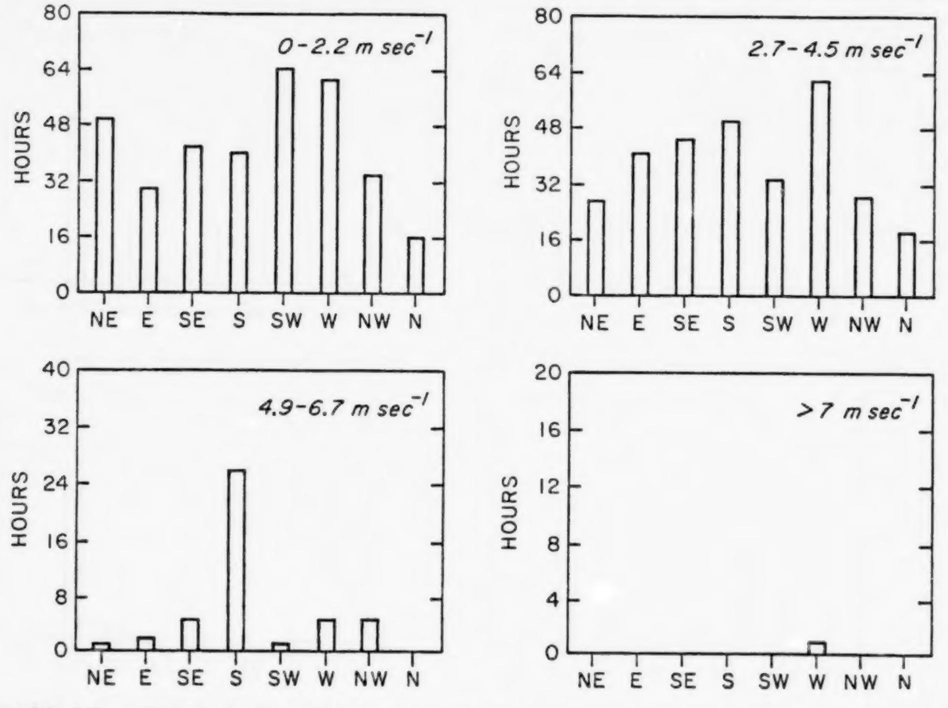


Figure 11.15. Histograms of wind speed and direction for ZAPS I, July, 1977.

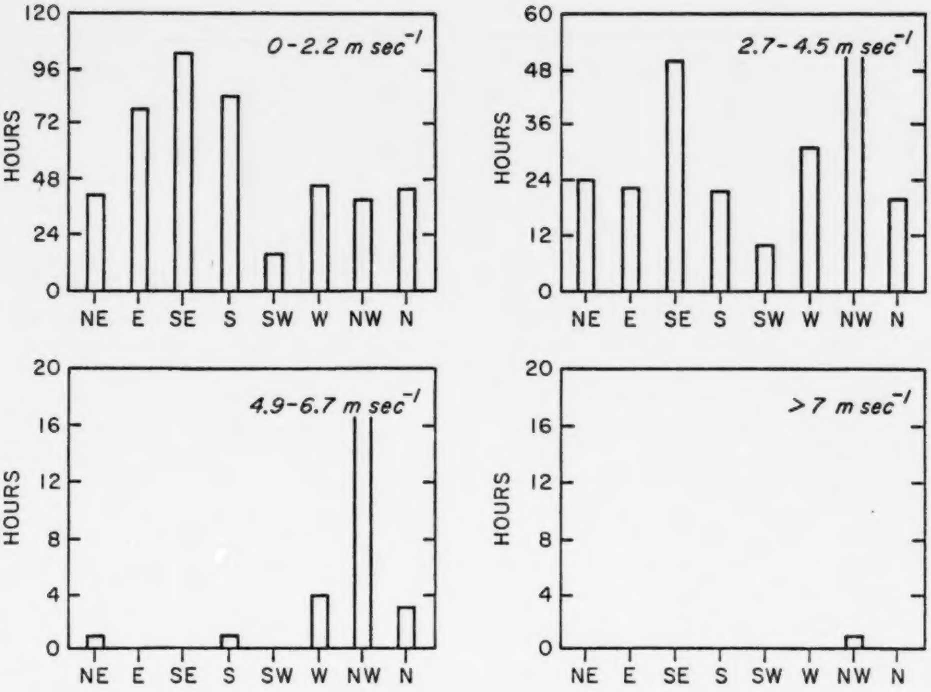


Figure 11.16. Histograms of wind speed and direction for ZAPS I, August, 1977.

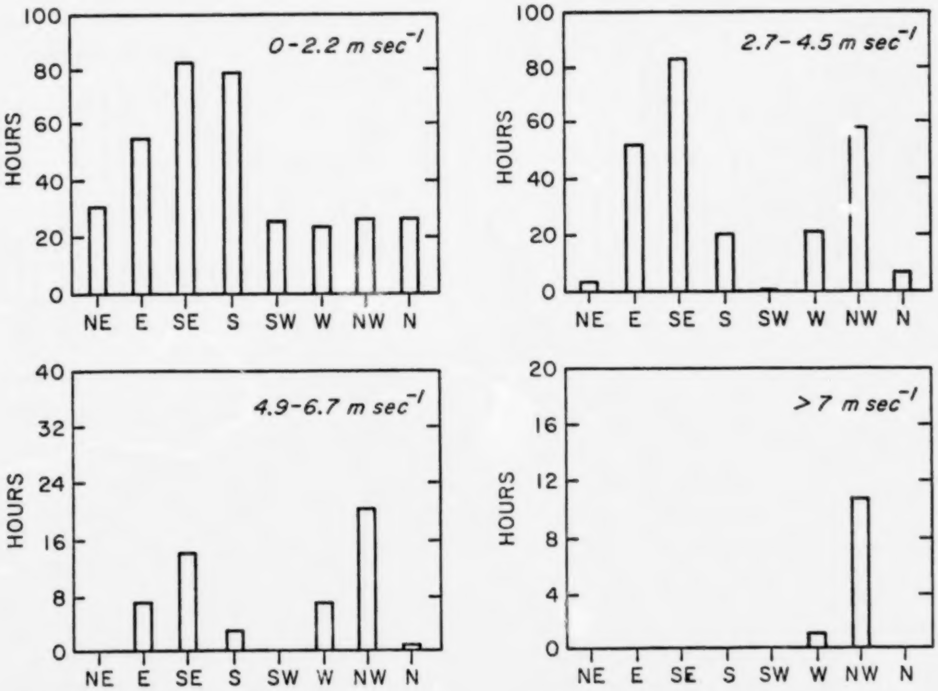


Figure 11.17. Histograms of wind speed and direction for ZAPS I, September, 1977.

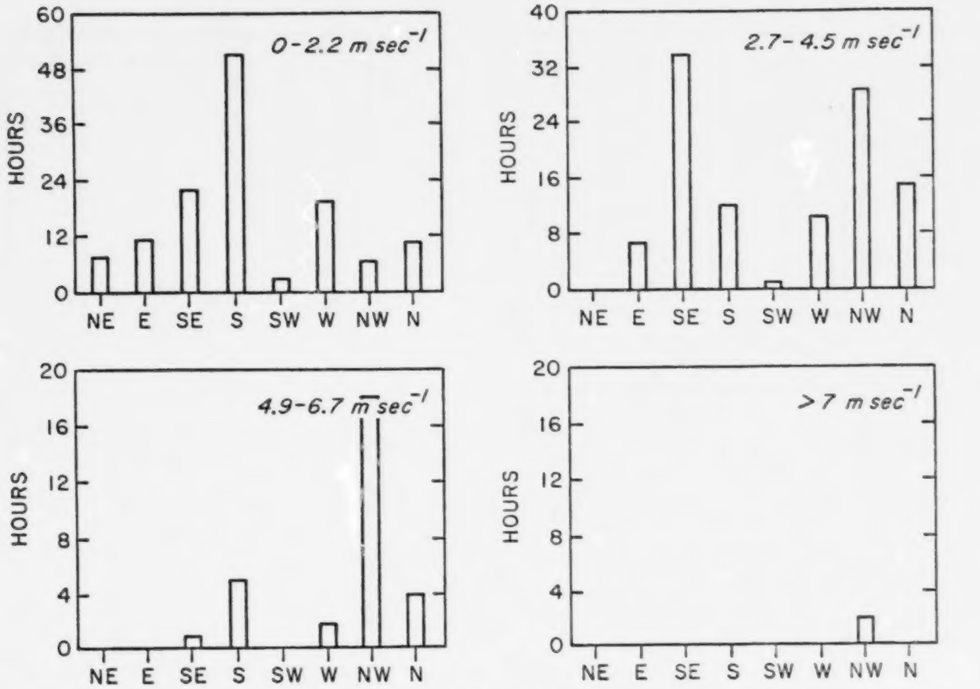


Figure 11.18. Histograms of wind speed and direction for ZAPS I, October, 1977.

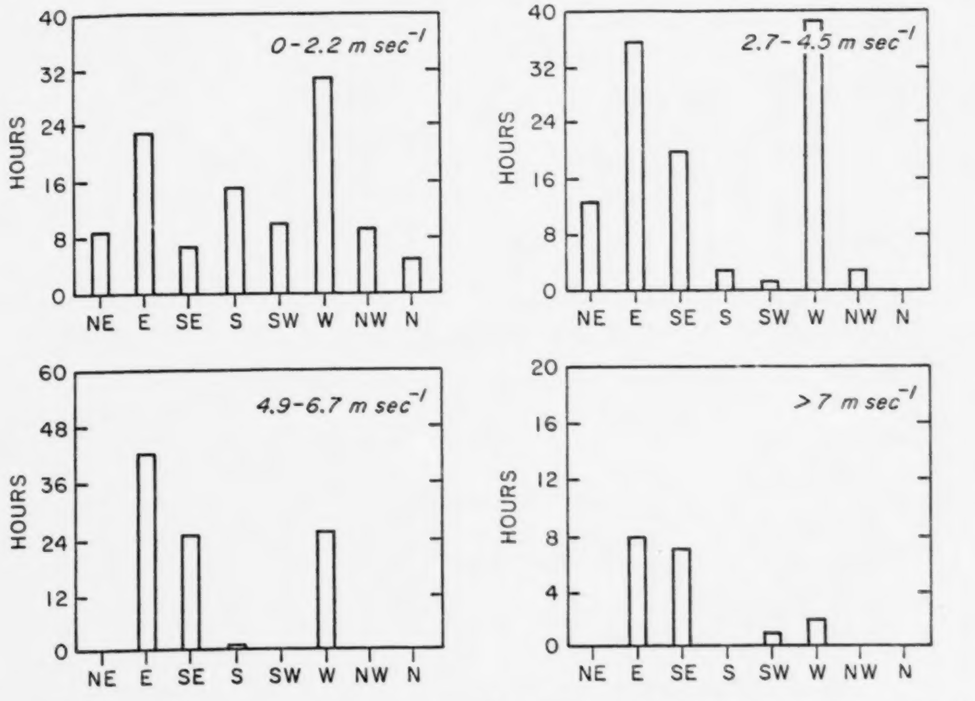


Figure 11.19. Histograms of wind speed and direction for ZAPS II, April, 1976.

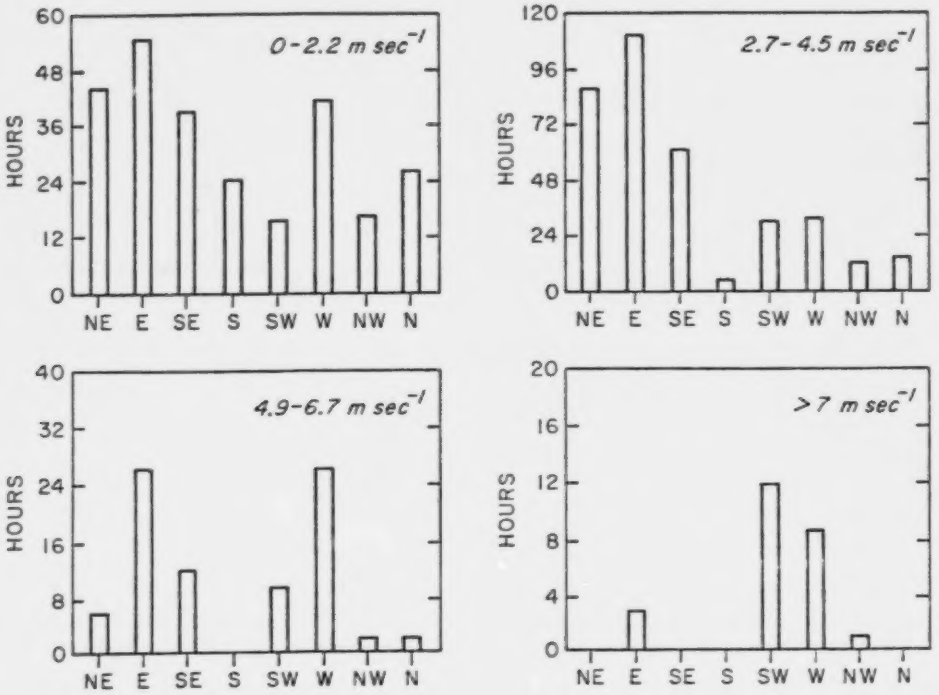


Figure 11.20. Histograms of wind speed and direction for ZAPS II, May, 1976.

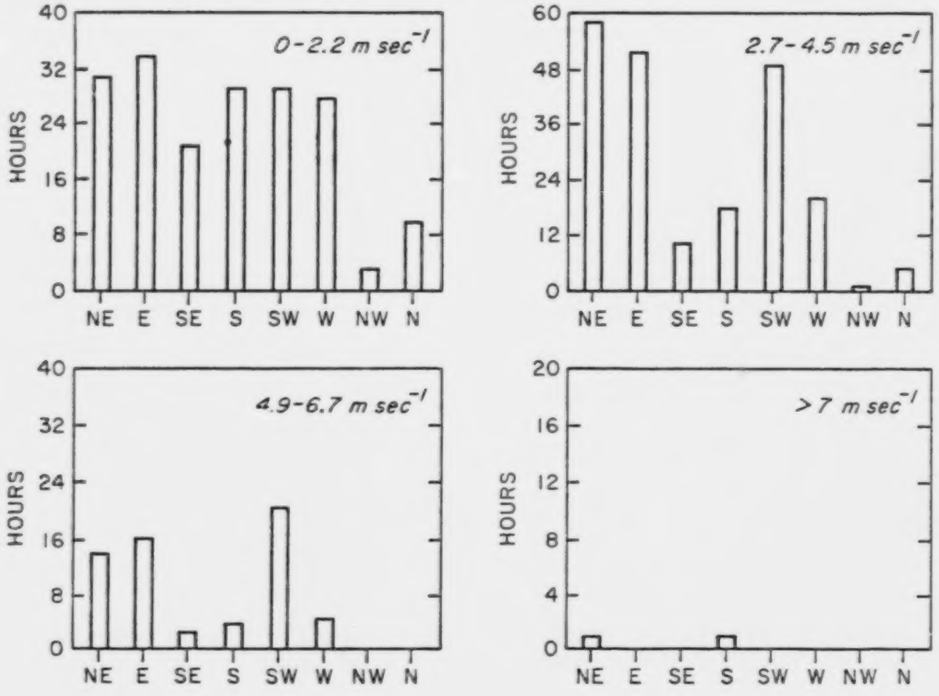


Figure 11.21. Histograms of wind speed and direction for ZAPS II, June, 1976.

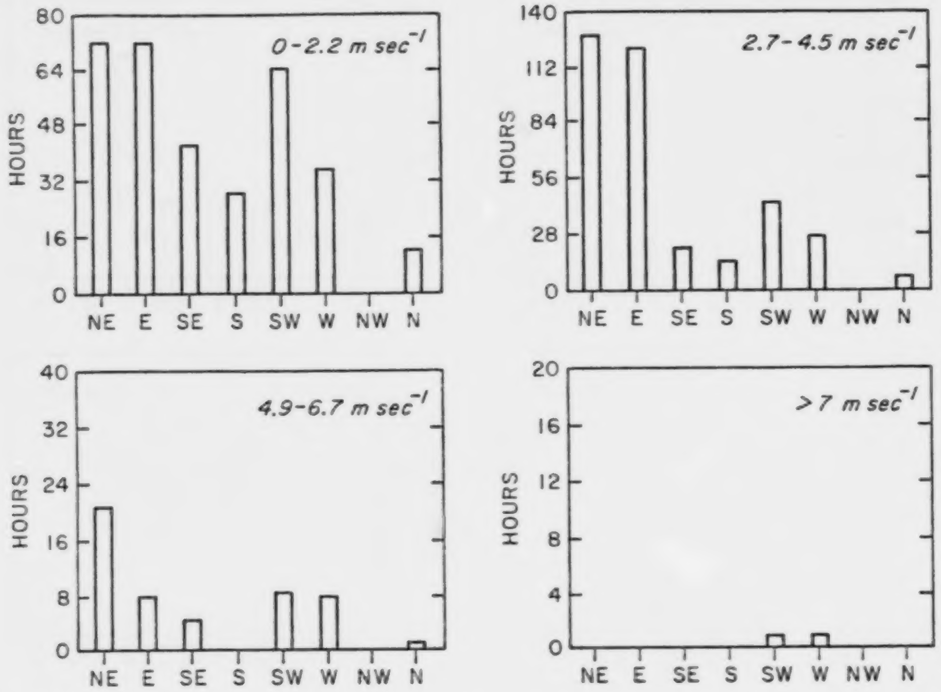


Figure 11.22. Histograms of wind speed and direction for ZAPS II, July. 1976.

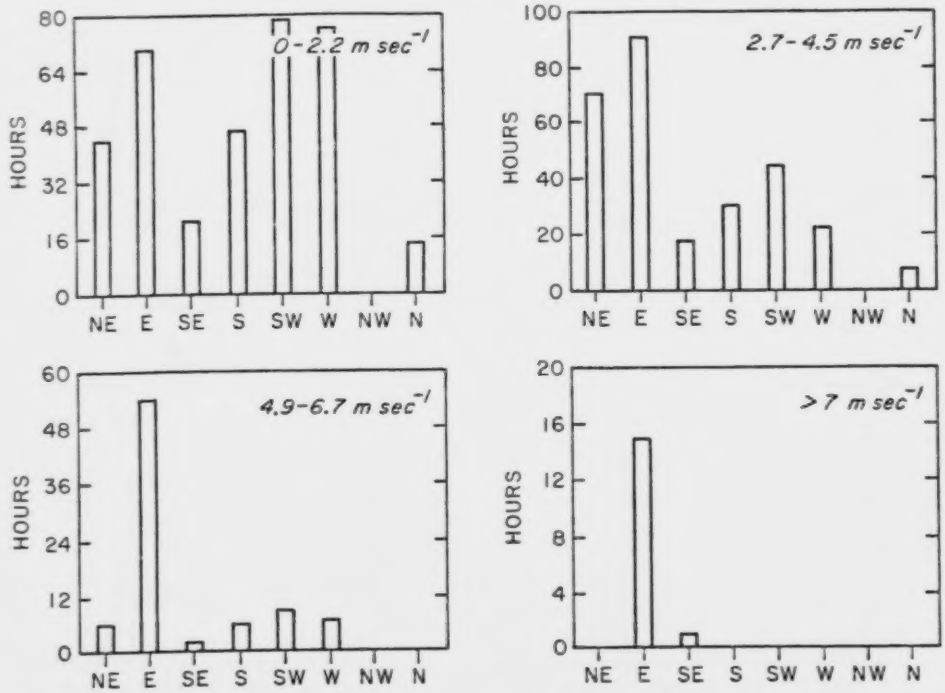


Figure 11.23. Histograms of wind speed and direction for ZAPS II, August, 1976.

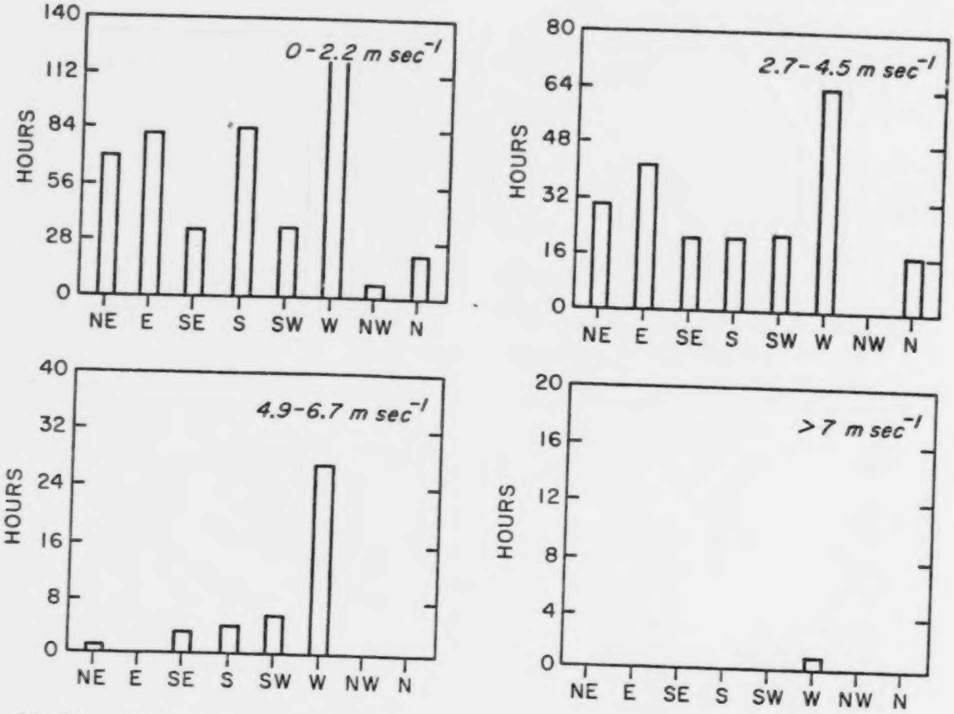


Figure 11.24. Histograms of wind speed and direction for ZAPS II, September, 1976.

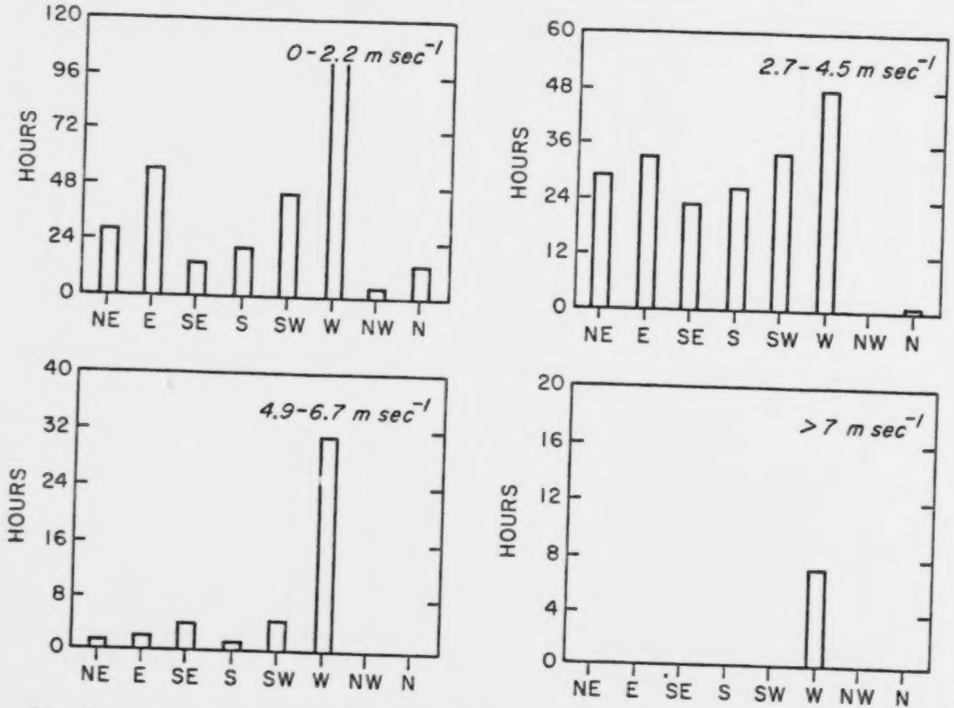


Figure 11.25. Histograms of wind speed and direction for ZAPS II, October, 1976.

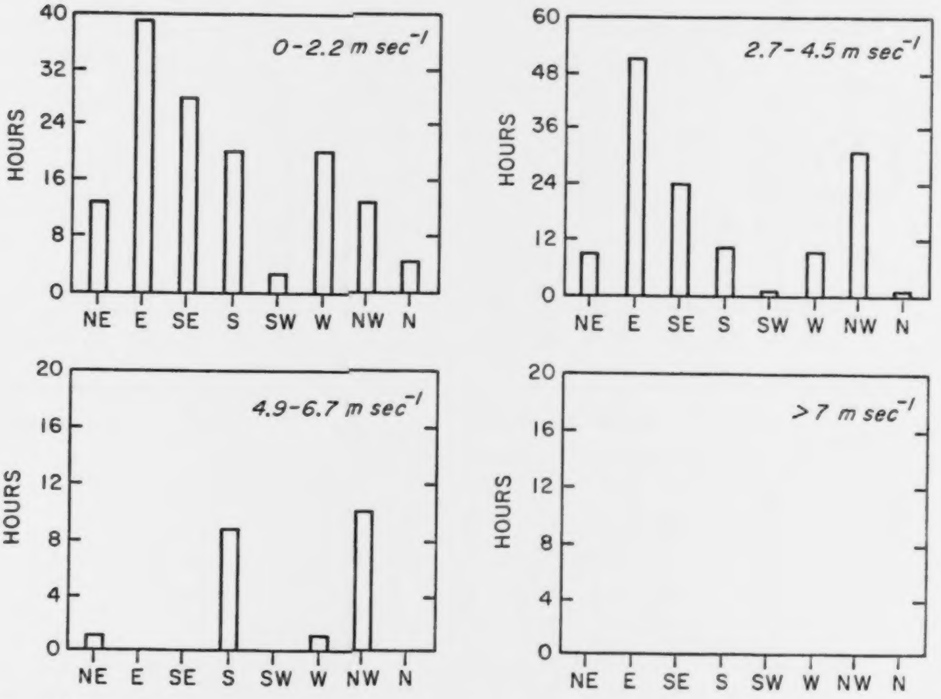


Figure 11.26. Histograms of wind speed and direction for ZAPS II, April, 1977.

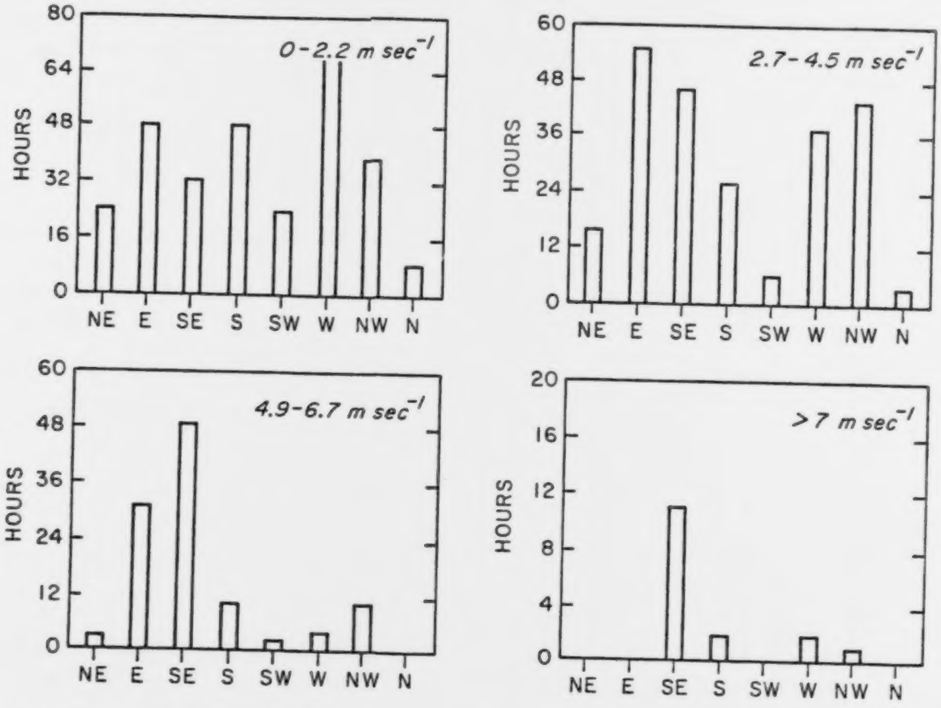


Figure 11.27. Histograms of wind speed and direction for ZAPS II, May, 1977.

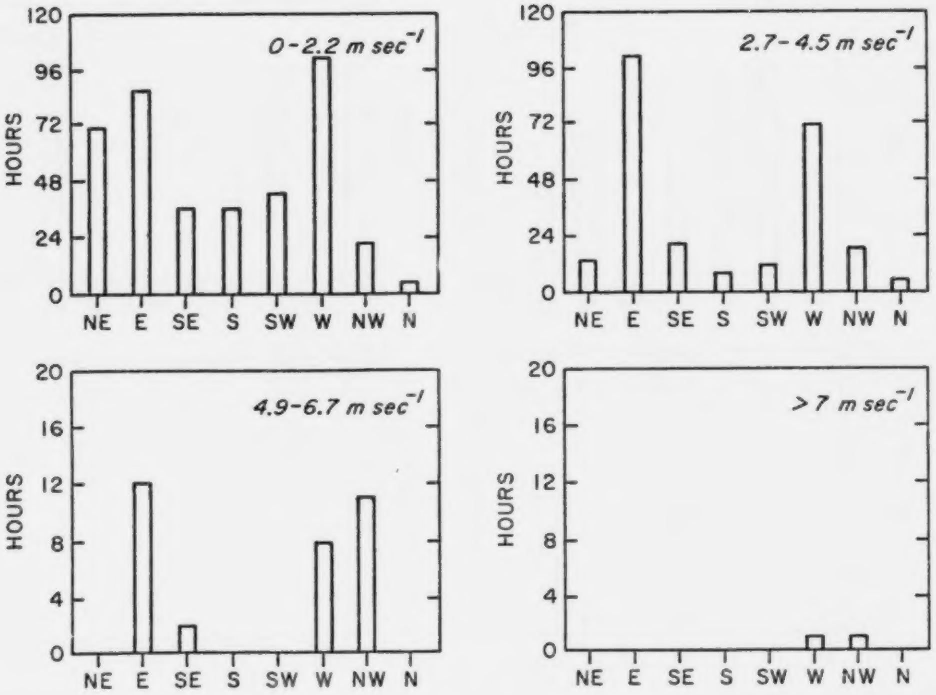


Figure 11.28. Histograms of wind speed and direction for ZAPS II, June, 1977.

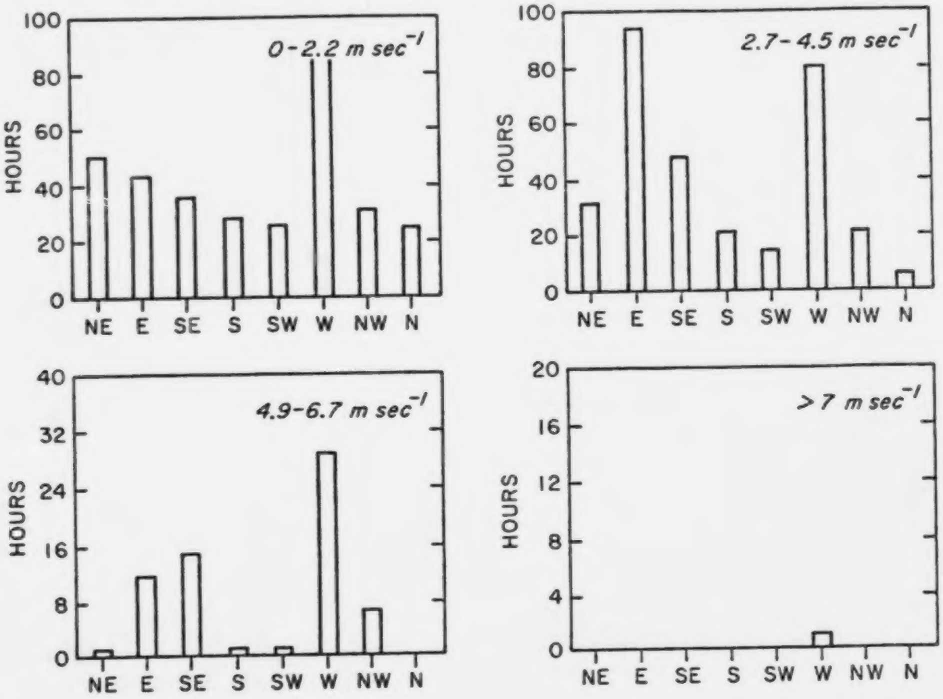


Figure 11.29. Histograms of wind speed and direction for ZAPS II, July, 1977.

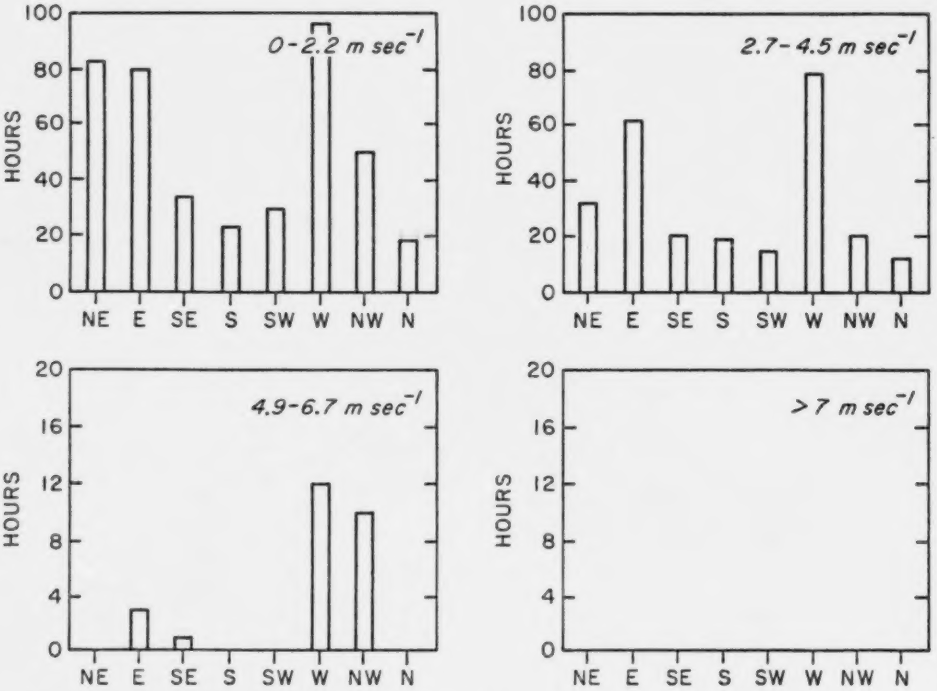


Figure 11.30. Histograms of wind speed and direction for ZAPS II, August, 1977.

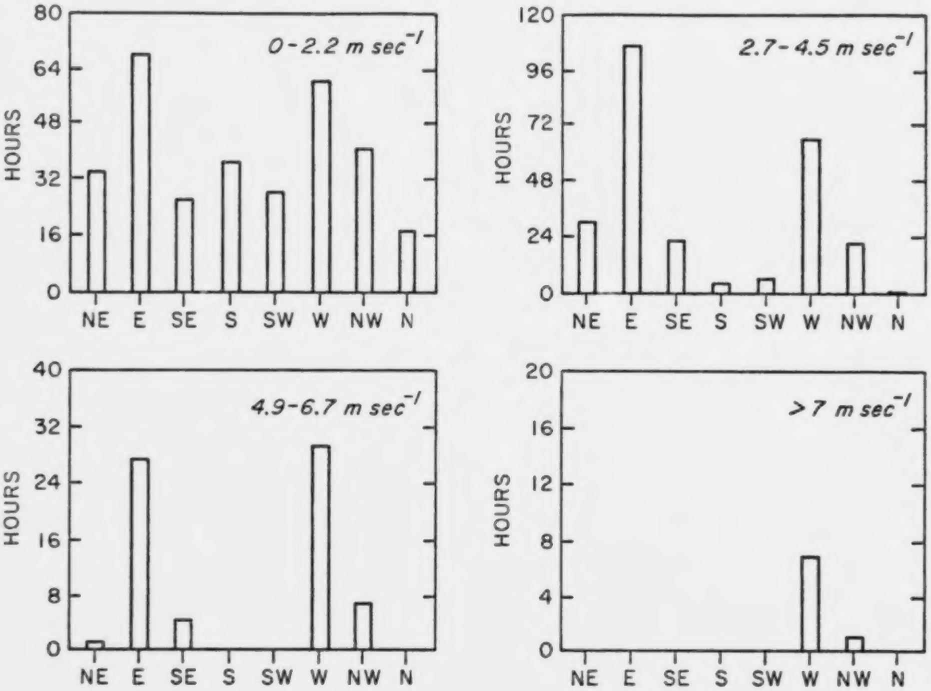


Figure 11.31. Histograms of wind speed and direction for ZAPS II, September, 1977.

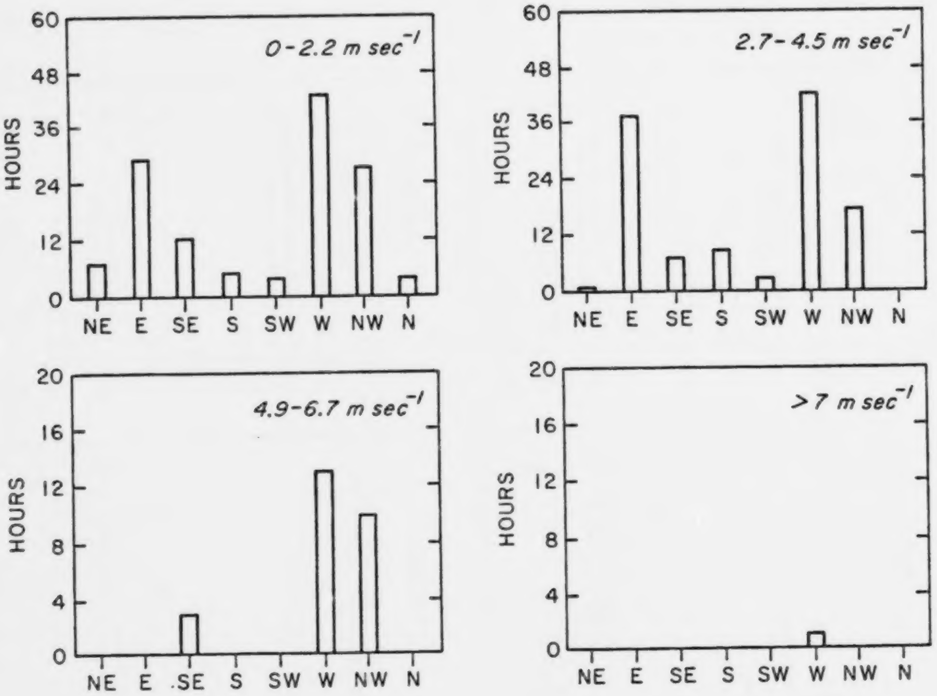


Figure 11.32. Histograms of wind speed and direction for ZAPS II, October, 1977.

SECTION 12

THE EFFECT OF SO_2 ON SOIL MICROORGANISM ACTIVITY

J.C. McFarlane, R.D. Rogers, and D.V. Bradley, Jr.

ABSTRACT

Soils from each of the Zonal Air Pollution Study (ZAPS) fumigation plots were analyzed for their hydrogen oxidation potential using *Alcaligenes paradoxus* as a representative microorganism. In the ZAPS I soils, there was a significant decrease in activity associated with increasing SO_2 insult. In the ZAPS II soils, no trend was present except that the microorganism activity in the highest SO_2 fumigation was significantly lower than all other plots. The results of these tests indicate that after 1 year of fumigation, only the most severe insult resulted in a detectable depression. However, after 2 years the soil microbiota was significantly affected by the SO_2 fumigation. These tests also indicate that the insult measurement technique employed might serve as a monitoring tool with sufficient sensitivity to detect this insult.

INTRODUCTION

In recent studies (McFarlane *et al.*, 1978a), it was found that the activity of the hydrogen-oxidizing microorganisms found in soils could be assayed very easily and accurately. One of the organisms, *Alcaligenes paradoxus*, (Rogers et al., 1978) was found to be one of the predominant hydrogen-oxidizing soil microorganisms. All soils tested thus far (McFarlane *et al.*, 1978b) have had an inherent hydrogen-oxidizing activity. In cell cultures these organisms can exist as either heterotrophs, which use the supplied organics or as autotrophs, which use the energy released in the enzymatic oxidation of elemental hydrogen. Their activity in natural soils varies with each soil type and is regulated presumably by the availability of either mineral or organic nutrients.

In laboratory experimentation (Rogers, 1978, personal communication), it has been shown that the rate of hydrogen oxidation is a sensitive indicator of the availability of mercury and cadmium in soil and solution. In these cases it appeared that the effect of the toxins was directly on the enzyme system since decreased hydrogenase activity was observed while no change in cell numbers was detected. This sensitivity to pollutants in the laboratory was the reason for testing the applicability of this biological monitor under conditions of insult in the field. The ZAPS study afforded an excellent test system where test plots were more or less uniform regarding soil type and pollutant concentration. Differences in the hydrogen oxidation activities determined by this assay are thought to represent an index of biochemical alterations in soil microorganisms. Laboratory experimentation with mercury and cadmium (unpublished) in solution and in soil have indicated that the hydrogen fixation activity indicates the insult of those pollutants. Tests are currently underway to determine if this test may be used as an index of the overall biological insult of a pollutant.

MATERIALS AND METHODS

Soils were collected in November 1976 from each of the ZAPS fumigation plots†. Composite samples of the top 5 cm were collected at random and mixed together for each treatment plot. In the laboratory the soils were mixed and the large rocks and pebbles were removed by screening the soil through a 2 mm soil sieve. The moisture content of the soils when they arrived was 5.0% ± 0.7% for the ZAPS I soils and 10.2% ± 4.9% for the ZAPS II soils. The waterholding capacity (WHC) of the soils at each site was similar; the WHC of ZAP I soils was 5.1 ± 0.1 ml/20 g and the WHC for the ZAPS II soils was 7.1 ± 0.4 ml/20 g.

Two hundred grams of each soil was placed in large petri dishes (15 cm) and sufficient water was added to bring these samples to 50% of their water holding capacities. The soils were incubated in 15 cm, closed petri dishes for 7 days at 30°C and the water content of each soil was adjusted as required. The incubation period was used to bring the microorganism populations into equilibrium.

Evaluation of the hydrogen oxidation potential of the soils was determined as follows. Twenty grams (equivalent dry weight) of soil was placed in each of seven, 1-liter round flasks which served as reaction vessels. Because it was critical to know the amount of water added to each reaction vessel, the water content of the soil was determined gravimetrically at the start of the activity test, and a second check on soil water content was made immediately prior to analysis. Initially, enough water was added to bring each sample to 140% of its water-holding capacity. The flasks were closed with rubber stoppers and the resultant soil slurry was spread over the inner surface by shaking.

Five millicuries of elemental tritium was obtained in a 1-liter cylinder pressurized with nitrogen. The specific activity of tritium in the cylinder was quantitatively determined by a test similar to the procedure used in these experiments (McFarlane *et al.*, 1978c). After the flasks had been flushed with air, 5 ml of elemental tritium (580 nCi) was injected through the rubber stopper with a gas-tight syringe. These bottles were then stored at 30° C for various periods of time before analysis. The reaction was stopped by opening the flask to allow the remaining elemental tritium to escape, and by adding 50 ml of benzene. The water was distilled in a benzene and water azeotrope (Moghissi *et al.*, 1973) and the amount of tritium recovered as water was determined by liquid scintillation counting.

---

† We thank Dr. John Taylor for collecting the soil samples.

The reaction rate was determined by analyzing replicate samples at various times. This produced a series of measurements which yielded curves that are described by a regression function known as the exponential growth model:

(1) $$Y - P_1 [1-\mathrm{EXP}\ (-P_2t)] \ +E$$

where: Y = the amount of elemental tritium (HT) converted to HTO at any time

P_1 = the asymptotic tritium content (nCi)

P_2 = the reaction rate parameter (hours^{-1})

t = time in hours

E = the error function, assumed to be Gaussian

Each data set was fit to this regression model using a non-linear least squares program.

The derivative of formula (1) with respect to time gives the velocity of the reaction:

(2) $$\frac{dy}{dt} = P_1P_2\ e^{-P_2t}$$

At time zero the velocity is maximal and equals P_1P_2. If the concentration of oxidized tritium is expressed in terms of percent or as a fraction, P_1 equals 1.0 and P_2 therefore equals the maximum velocity of the reaction. The dimensions in these tests were in units of the fraction of tritium converted per hour.

RESULTS AND DISCUSSION

The results of these tests generated a family of curves (Figure 12.1) which represent different rates of tritium oxidation. The maximum velocities occurred at T = 0. In these experiments, the concentration of atmospheric hydrogen was assumed to be 0.50 ppm (volume:volume) (Ehhalt et al., 1975) and was the dominant source of elemental hydrogen. The tritium source also contained elemental hydrogen since in the preparation H_2 was used for the first dilution. This amounted to 10.5 ppm of hydrogen in the tritium bottle and resulted in an additional 0.05 ppm of hydrogen in each 1-liter flask.

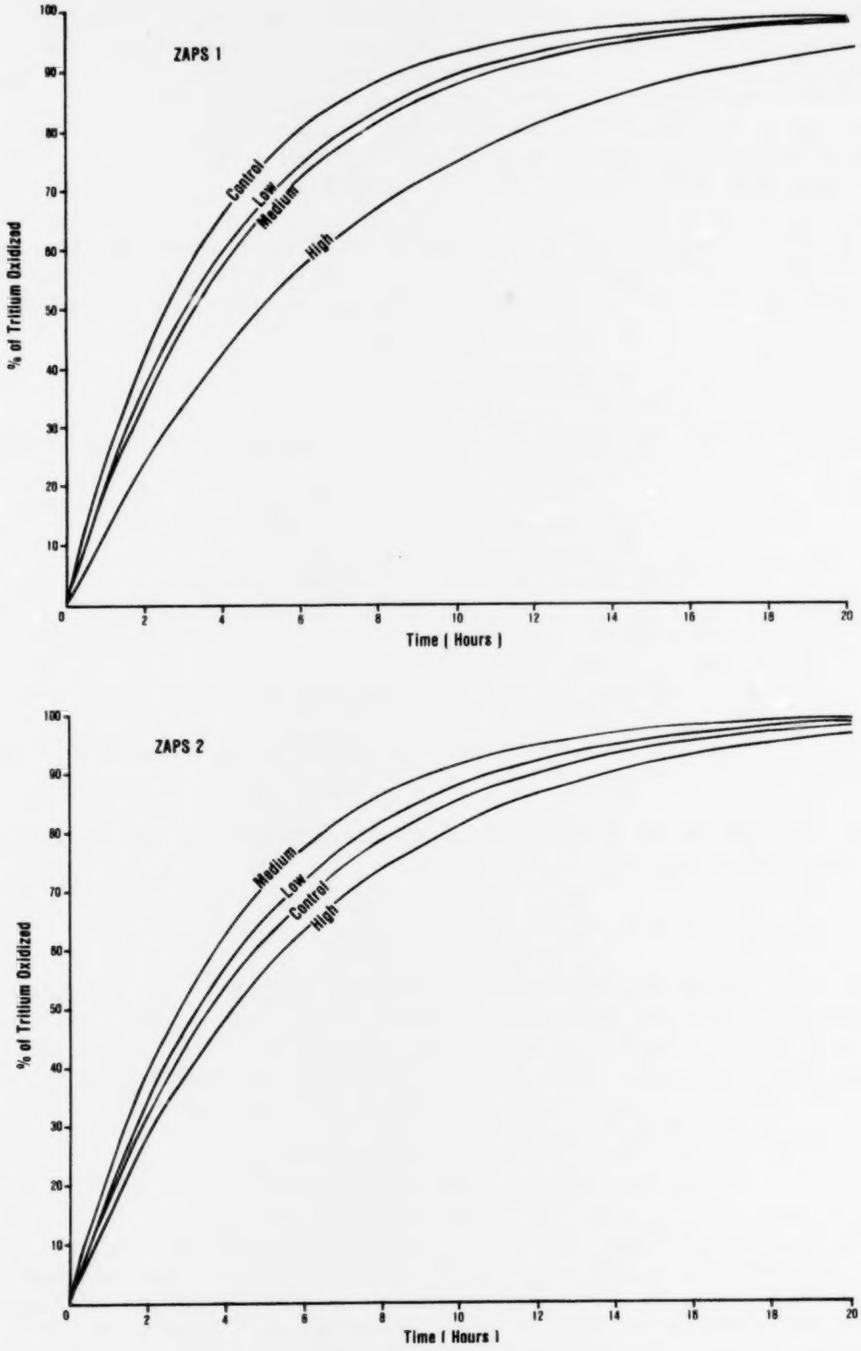


Fig. 12.1 Percent of elemental tritium oxidized by soil microorganisms

Since tritium was present in trace concentrations, the oxidation reactions primarily involved elemental hydrogen and the concentration of atmospheric hydrogen determined the original or maximum velocity. These rates are therefore similar to those expected under field conditions which favor soil microorganism activity.

Maximum velocities were calculated for each study plot and are presented in Table 12.1. In the ZAPS I soils (2 years of fumigation) there was a decreasing gradient in microorganism activity which was correlated with increasing SO_2 insult. In the ZAPS II soils the same pattern was not evident. Variability was higher in the rate analyses for the ZAPS II soils and therefore no statistical differences were evident for the microorganism activity in the lower SO_2 concentrations. Nevertheless, there was a significant difference between the high and low SO_2 treatments.

TABLE 12.1. MAXIMUM HYDROGEN OXIDATION RATES BY SOIL MICROORGANISMS AFTER VARIOUS SO_2 FUMIGATION TREATMENTS

Treatment Designation	P_2 = Vmax (% oxidized/hour)	
ZAPS I		
Control	28	a*
Low	23	b
Medium	21	b
High	14	c
ZAPS II		
Control	20	x
Low	21	x
Medium	25	x
High	17	y

*Letters represent groupings which are significantly different using Duncan's multiple range test.

During collection, transport, screening, mixing and pretreatment incubation, ample opportunity was given for these soils to return to equilibrium with environmental conditions. The residual effect from SO_2 fumigation is therefore surprising. The reason for this correlation between the microorganism activity and SO_2 insult is unknown. It is tempting to postulate that alterations in dissolved salts, pH or other factors were responsible. However, when some of the physical parameters of these soils (Table 12.2) were examined no obvious correlations were evident. The possibility obviously exists that the correlations are an accidental artifact and were not associated with SO_2.

The lack of correlation between the three lowest SO_2 fumigations with microorganism activity in the ZAPS II soils may have resulted from the lack of effect after only 1 year of treatment or it might indicate random soil sampling error. On the other hand, the pattern of activity observed in ZAPS I and between the high SO_2 level and all lower fumigation levels in ZAPS II suggest that this test may serve as an important bio-indicator of perturbation. Thus, although this study was not conclusive proof, it is nevertheless a strong indication that additional study is warranted.

TABLE 12.2. SOIL CHARACTERISTICS FOR THE ZAPS I AND ZAPS II SITES

	ZAPS I				ZAPS II			
Plots	A	B	C	D	A	B	C	D
Organic Matter	1.79	1.77	1.58	1.33	2.59	2.00	1.96	2.94
pH in 0.01 $mCaCl_2$	5.3	6.6	6.8	6.4	6.8	5.9	7.1	7.0
pH in H_2O	6.5	7.2	7.0	6.9	7.2	6.8	7.3	7.4
Electrical Conductivity 1:5 in H_2O (x 10^3 m mho)	0.12	0.20	--	0.39	0.45	0.15	--	0.52
Settling volume of 15-g soil (ml)	19.0	20.0	19.0	18.0	22.0	23.0	23.0	24.0
Exchangeable Al (meq/100 g)	0	0	0	0	0	0	0	0
Exchangeable H (meq/100 g)	0	0	0	0	0	0	0	0
Extractable cations in $BaCl_2$ (meq/100 g):								
Na	0.05	0.19	0.08	0.08	0.37	0.08	0.08	0.51
K	0.71	1.16	0.53	0.58	1.50	1.24	0.77	1.53
Mg	2.18	2.74	3.20	2.89	4.44	4.04	3.94	5.25
Ca	3.57	6.20	8.59	6.20	11.76	8.54	13.58	15.28
Total	6.51	10.29	12.37	9.75	18.07	13.90	18.37	22.57
Particle Size (%):								
Sand	47.0	49.9	45.5	41.9	29.8	32.6	24.2	24.7
Silt	37.7	36.7	37.3	41.8	38.0	40.4	49.6	52.9
Clay	15.3	13.4	17.2	16.3	32.0	27.0	28.2	22.4
Texture	L	L	L	L	CL	L to CL	L to Silt	Silt

REFERENCES

Ehhalt, D.H., L. E. Hiedt, R. H. Lueb and W. Pollock. 1975. The Vertical Distribution of Trace Gases in the Stratosphere. Pageoph, 113:389-402.

McFarlane, James C., Robert D. Rogers, and Donald V. Bradley. 1978a. Tritium Oxidation in Surface Soils. Environmental Science and Technology (accepted for publication).

McFarlane, J.C., R.D. Rogers, and D.V. Bradley, Jr. 1978b. Tritium Oxidation in Surface Soils--A Survey of Soils Near Five Nuclear Fuel Reprocessing Plants (in review).

McFarlane, J.C., R.D. Rogers and D.V. Bradley, Jr. 1978c. Elemental Tritium Analysis by Bio-Oxidation (in review).

Moghissi, A.A., E. W. Bretthauer, and E. H. Compton. 1973. Separation of Water from Biological and Environmental Samples for Tritium Analysis. Analytical Chemistry, 45:1565-1566.

Rogers, R.D., D.V. Bradley and J.C. McFarlane. 1978. The Role of Hydrogen-Oxidizing Microorganism, *Alcaligenes paradoxus*, in Environmental Tritium Oxidation (in review).

SECTION 13

EFFECTS OF CHRONIC LOW LEVEL SO_2 EXPOSURE ON PRODUCERS AND LITTER DYNAMICS

J. L. Dodd, W. K. Lauenroth, G. L. Thor and M. B. Coughenour

ABSTRACT

The effects of controlled levels of SO_2 on producers and litter dynamics of a Montana grassland were investigated for three consecutive growing seasons (1975-1977). The main findings of the study were that net primary productivity was apparently not reduced; the dominant grass, *Agropyron smithii*, showed premature leaf senescence and possibly increased growth rates; litter dynamics were apparently not affected; nutritional quality was reduced; assimilation and distribution of ^{35}S and ^{14}C are discussed.

INTRODUCTION

This section consists of a discussion of the research progress made by a group of scientists at Colorado State University investigating the effects of controlled levels of SO_2 on a northern Great Plains grassland. The section is organized by subsection and within each subsection an attempt is made to document the progress in a given research area that has been made since reporting the first year (1975) results in Dodd *et al.* (1978). We have also included certain information in appendix form to make it available for use by other investigators on the Colstrip coal-fired power plant project. Much of the information included herein has received only preliminary analysis and should therefore be interpreted with caution.

ABOVEGROUND PLANT BIOMASS DYNAMICS AND NET PRIMARY PRODUCTIVITY

The effect of controlled levels of SO_2 fumigation on biomass dynamics and aboveground net primary productivity was studied at the two ZAPS sites. The harvest method, as described by Lauenroth *et al.* (1975), was utilized in collecting biomass data. In 1976 the size of the circular quadrats was changed from 0.5 to 0.25 m^2, and the number of quadrats clipped per treatment was increased from 10 to 20.

The seasonal dynamics of biomass for all species combined (Figure 13.1) were quite similar across treatments for the ZAPS I site in 1975 and 1976. The ZAPS II site, which was initiated in 1976, showed a slightly lower rate of net productivity on the control treatment than on the SO_2 treatments. The control plot probably had lower productivity because cattle had grazed it more in the years preceding the current experiment than they had the three treated plots. This differential grazing pressure likely resulted from the fact that the control plot was much closer to a corner livestock gate than were the treated plots. If heavier grazing pressure were applied to the control treatment, we would expect a lower level of net primary production in the first few years after exclusion of the livestock. Eventually the successional process should return the control plot to the same level of productivity as the treated plots. Unfortunately, this is the same direction of change that we might expect if the SO_2 treatments depress productivity. Another possible explanation for the difference in productivity between the control and the SO_2-treated plots is related to soils. It was stated earlier (Section 11) that soils of the control are somewhat different than those of the treated plots. We do not know if these differences and differences in soil water content (Section 11) are sufficient to account for the productivity differences. Care must be taken in interpreting future changes on these study plots.

The major contributors to total biomass on both ZAPS sites are cool season grasses, and as expected, the trend of biomass production for cool season grasses (Figure 13.2) closely follows the trend for total biomass. There does not appear to be any correlation between SO_2 treatment and seasonal trend for this group of plants.

The other major contributors to total biomass are the cool season forbs (Figure 13.3). Again, there does not appear to be any treatment effect. Warm season grasses and half shrubs also do not appear to respond in any clear manner to the treatments (Figures 13.4 and 13.5).

The two most important cool season grasses on our study sites are western wheatgrass and prairie June grass. The seasonal dynamics for western wheatgrass (Figure 13.6) closely follow those for cool season grasses since it is the major contributor to that category. Because of its dominance on the ZAPS sites and in the northern Great Plains, we are particularly interested in any possible response by western wheatgrass to the treatments. Treatment effects were not detectable on either ZAPS site for any of the years studied.

Prairie June grass (Figure 13.7) shows a trend on ZAPS I for 1975 and 1976 in which the growth rate during the major growth period (April through June) is greatest on the low treatment, intermediate on the control and medium treatment, and lowest on the high treatment. However, the ZAPS II site reveals a reversal of that trend, indicating that this is probably due to variation on the sites rather than a response to treatment.

changed from 0.5 to 0.25 m^2, and the number of quadrats clipped per treatment was increased from 10 to 20.

The seasonal dynamics of biomass for all species combined (Figure 13.1) were quite similar across treatments for the ZAPS I site in 1975 and 1976. The ZAPS II site, which was initiated in 1976, showed a slightly lower rate of net productivity on the control treatment than on the SO_2 treatments. The control plot probably had lower productivity because cattle had grazed it more in the years preceding the current experiment than they had the three treated plots. This differential grazing pressure likely resulted from the fact that the control plot was much closer to a corner livestock gate than were the treated plots. If heavier grazing pressure were applied to the control treatment, we would expect a lower level of net primary production in the first few years after exclusion of the livestock. Eventually the successional process should return the control plot to the same level of productivity as the treated plots. Unfortunately, this is the same direction of change that we might expect if the SO_2 treatments depress productivity. Another possible explanation for the difference in productivity between the control and the SO_2-treated plots is related to soils. It was stated earlier (Section 11) that soils of the control are somewhat different than those of the treated plots. We do not know if these differences and differences in soil water content (Section 11) are sufficient to account for the productivity differences. Care must be taken in interpreting future changes on these study plots.

The major contributors to total biomass on both ZAPS sites are cool season grasses, and as expected, the trend of biomass production for cool season grasses (Figure 13.2) closely follows the trend for total biomass. There does not appear to be any correlation between SO_2 treatment and seasonal trend for this group of plants.

The other major contributors to total biomass are the cool season forbs (Figure 13.3). Again, there does not appear to be any treatment effect. Warm season grasses and half shrubs also do not appear to respond in any clear manner to the treatments (Figures 13.4 and 13.5).

The two most important cool season grasses on our study sites are western wheatgrass and prairie June grass. The seasonal dynamics for western wheatgrass (Figure 13.6) closely follow those for cool season grasses since it is the major contributor to that category. Because of its dominance on the ZAPS sites and in the northern Great Plains, we are particularly interested in any possible response by western wheatgrass to the treatments. Treatment effects were not detectable on either ZAPS site for any of the years studied.

Prairie June grass (Figure 13.7) shows a trend on ZAPS I for 1975 and 1976 in which the growth rate during the major growth period (April through June) is greatest on the low treatment, intermediate on the control and medium treatment, and lowest on the high treatment. However, the ZAPS II site reveals a reversal of that trend, indicating that this is probably due to variation on the sites rather than a response to treatment.

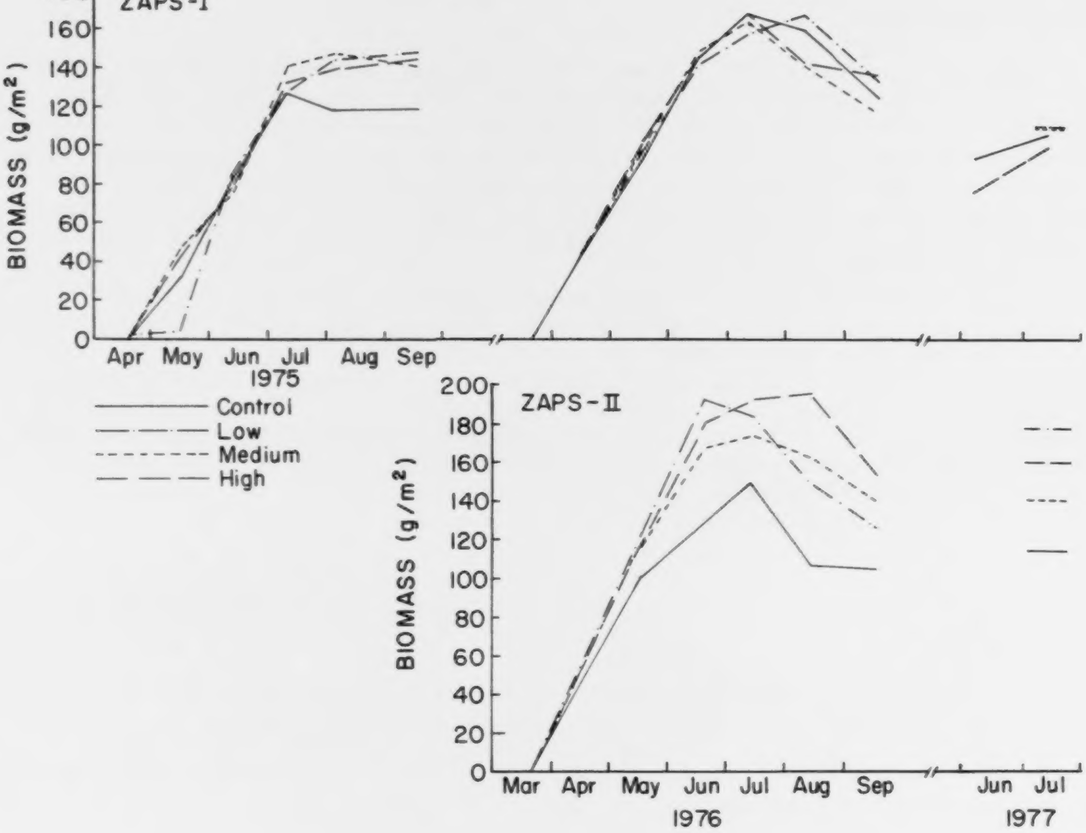


Figure 13.1. Seasonal change in current years production for all species combined, ZAPS I and II, 1975-1977.

Net primary productivity is presented in Table 13.1. We sampled only once in 1977, between 10 and 15 July, but are making the assumption that this sampling was close to the occurrence of the peak biomass for the year. This assumption is based on the timing of peak biomass for 1976. For 1975 and 1976, peak current production figures cited are the sums of peaks for the various functional groups based on frequent harvest data.

Examination of the data for total aboveground net production peaks does not show any treatment effect. We do see a change in production figures for each site over the years with an increase occurring from 1975 to 1976 and then a reduction between 1976 and 1977. The yearly trends in the functional groups generally reflect that trend. The interseasonal changes are due to year-to-year fluctuations in precipitation and the subsequent availability of soil water. Both 1975 and 1976 were wetter than normal years, especially in May and June, while 1977 was drier than normal (Section 11).

TABLE 13.1. PEAK STANDING CROPS OF CURRENT PRODUCTION AND ABOVEGROUND NET PRODUCTION*

Functional groups	ZAPS I Control	ZAPS I Low	ZAPS I Medium	ZAPS I High	ZAPS II Control	ZAPS II Low	ZAPS II Medium	ZAPS II High
Cool season grasses								
1975	104.4 ± 14.1	126.7 ± 16.1	120.3 ± 11.5	98.4 ± 17.2	131.4 ± 7.1	175.0 ± 17.4	148.9 ± 11.3	179.0 ± 12.9
1976	133.4 ± 12.0	130.6 ± 10.5	122.7 ± 7.6	109.4 ± 10.7	98.3 ± 6.9	154.1 ± 11.6	115.4 ± 8.9	136.6 ± 11.5
1977	77.0 ± 8.5	79.5 ± 12.2	80.0 ± 6.5	59.1 ± 5.2				
Warm season grasses								
1975	[illegible].6 ± 3.7	9.5 ± 2.9	8.4 ± 2.7	12.3 ± 4.8	8.0 ± 3.9	0	7.5 ± 4.5	3.5 ± 3.0
1976	6.1 ± 3.0	15.1 ± 5.0	5.1 ± 2.0	10.3 ± 3.7	0.1 ± 0.0	0	0.1 ± 0.1	2.6 ± 2.0
1977	4.4 ± 1.6	1.6 ± 0.8	1.4 ± 0.6	1.2 ± 0.5				
Cool season forbs								
1975	24.6 ± 3.3	15.0 ± 2.3	17.0 ± 2.6	35.7 ± 8.5	16.0 ± 2.8	30.0 ± 6.1	21.1 ± 4.9	16.9 ± 5.2
1976	31.6 ± 5.0	27.0 ± 3.7	33.6 ± 4.7	51.9 ± 6.8	11.5 ± 2.1	22.8 ± 4.0	23.7 ± 4.2	17.6 ± 4.3
1977	23.5 ± 3.7	24.5 ± 3.9	28.4 ± 5.0	31.1 ± 2.4				
Warm season forbs								
1975	1.2 ± 0.6	3.8 ± 2.6	5.3 ± 4.3	5.3 ± 5.1	0	0	0	0
1976	1.9 ± 1.5	2.4 ± 1.0	5.3 ± 2.8	7.2 ± 2.9	0	1.4 ± 1.4	0	0
1977	1.8 ± 1.7	5.1 ± 2.2	0.3 ± 0.3	5.9 ± 2.5				
Half shrubs								
1975	1.9 ± 1.8	0	14.9 ± 10.7	17.1 ± 12.3	2.2 ± 2.2	2.0 ± 2.0	3.6 ± 2.4	3.1 ± 1.3
1976	6.7 ± 6.7	0	4.3 ± 2.6	6.6 ± 4.7	5.0 ± 2.4	0	1.9 ± 1.0	3.1 ± 2.5
1977	0.3 ± 0.3	0	0.6 ± 0.4	3.7 ± 2.6				
Aboveground net production								
1975	138.7	155.0	165.9	168.8	157.6	207.0	181.1	202.5
1976	179.7	175.1	171.0	185.4	114.9	178.3	141.1	159.9
1977	107.0	110.7	110.7	101.0				

*Peaks for 1975 and 1976 were determined from frequent harvest data (see biomass dynamics discussion) while peaks for 1977 were from a single mid-July harvest date ($\bar{X}$ ± SE, $g \cdot m^{-2}$).

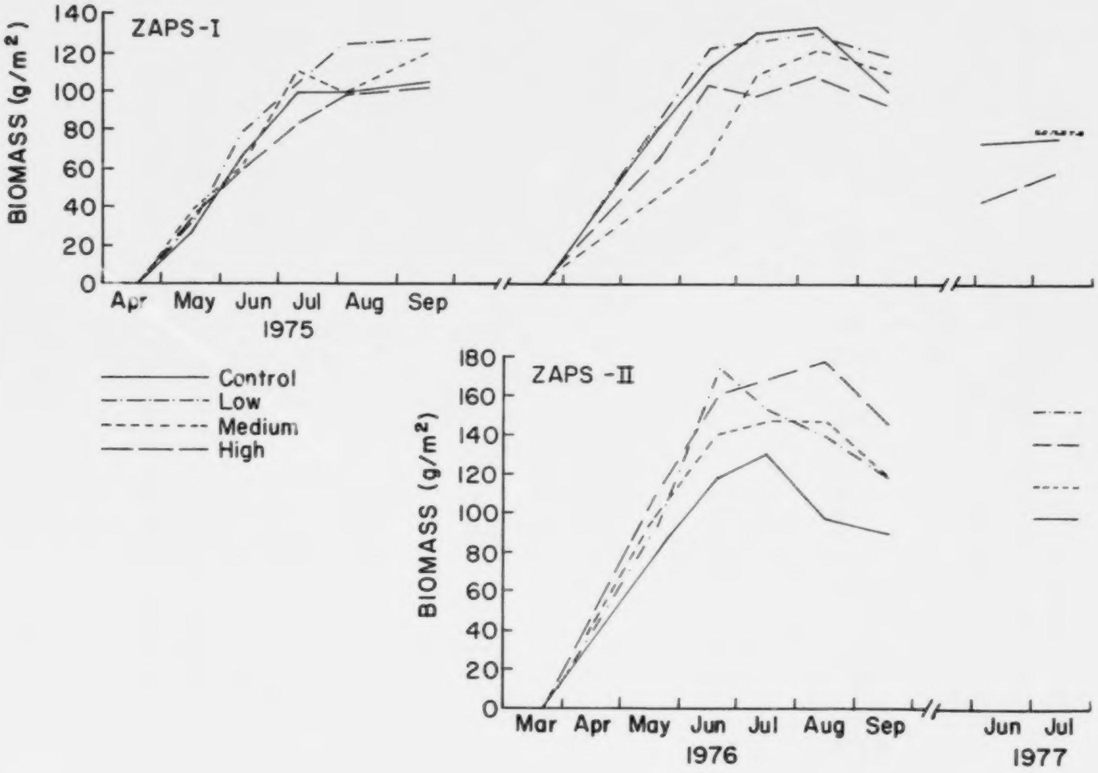


Figure 13.2. Seasonal change in current years production of cool season grasses, ZAPS I and II, 1975-1977.

Cool season grass production shows an interesting pattern on ZAPS I in 1976 and 1977. Peak values appear to be inversely related to SO_2 concentrations; however, the pattern was not repeated on ZAPS II. Cool season forbs showed a reversed trend in which peak values increased with SO_2 concentrations. Again, ZAPS II did not reveal the same trend. Based on the data we have collected thus far, we must conclude that no detectable treatment effects have occurred.

BELOWGROUND PLANT BIOMASS DYNAMICS

Crowns (mostly the subterranean portions of stem bases), roots, and rhizomes are the belowground organs of plants and are the principal storage organs for herbaceous plant species. It is, therefore, important to know what the response of this part of the producer system is to SO_2 stress. Unfortunately, state-of-the-art sampling techniques do not provide a satisfactory way to sample the living portion of this critical compartment. Therefore, we have measured dynamics of these components on a live-plus-dead

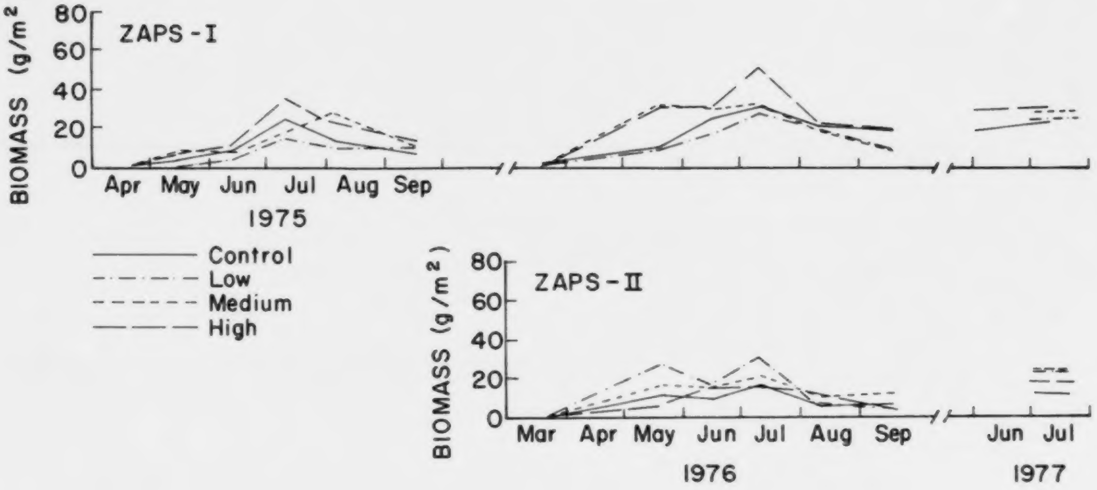


Figure 13.3. Seasonal change in current years production for cool season forbs, ZAPS I and II, 1975-1977.

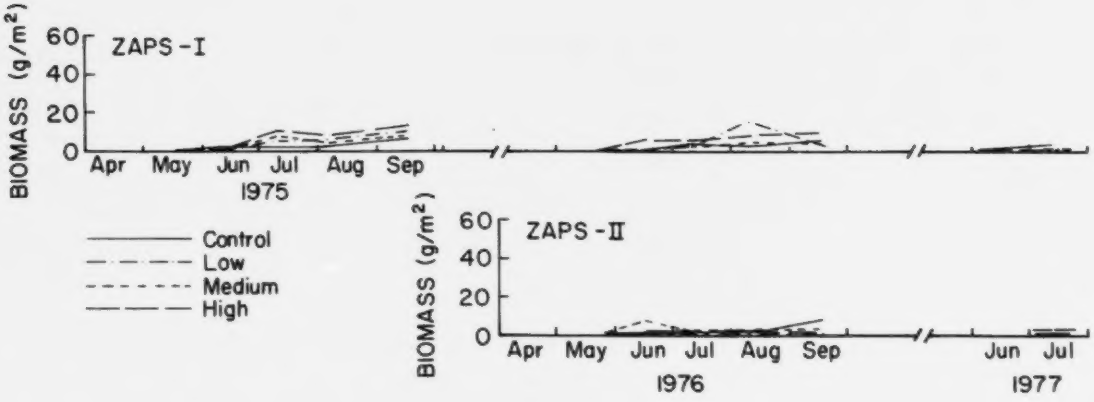


Figure 13.4. Seasonal change in current years production of warm season grasses, ZAPS I and II, 1975-1977.

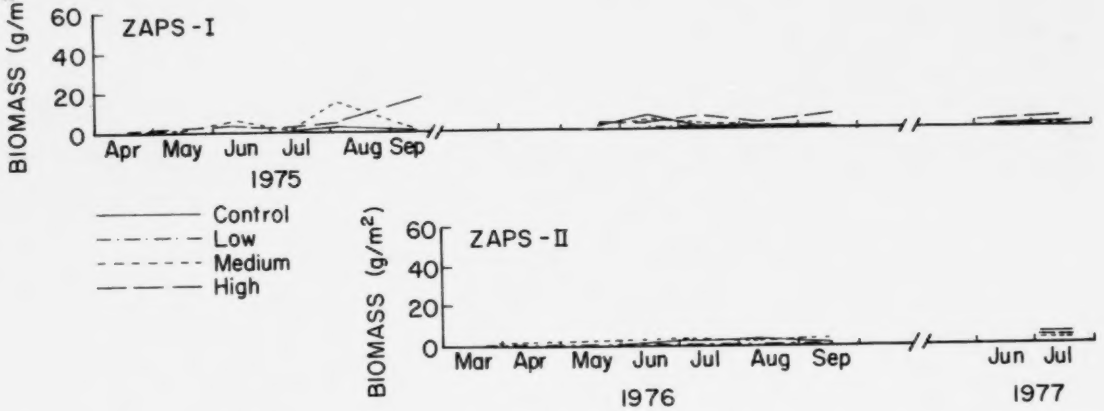


Figure 13.5. Seasonal change in current years production of half shrubs, ZAPS I and II, 1975-1977.

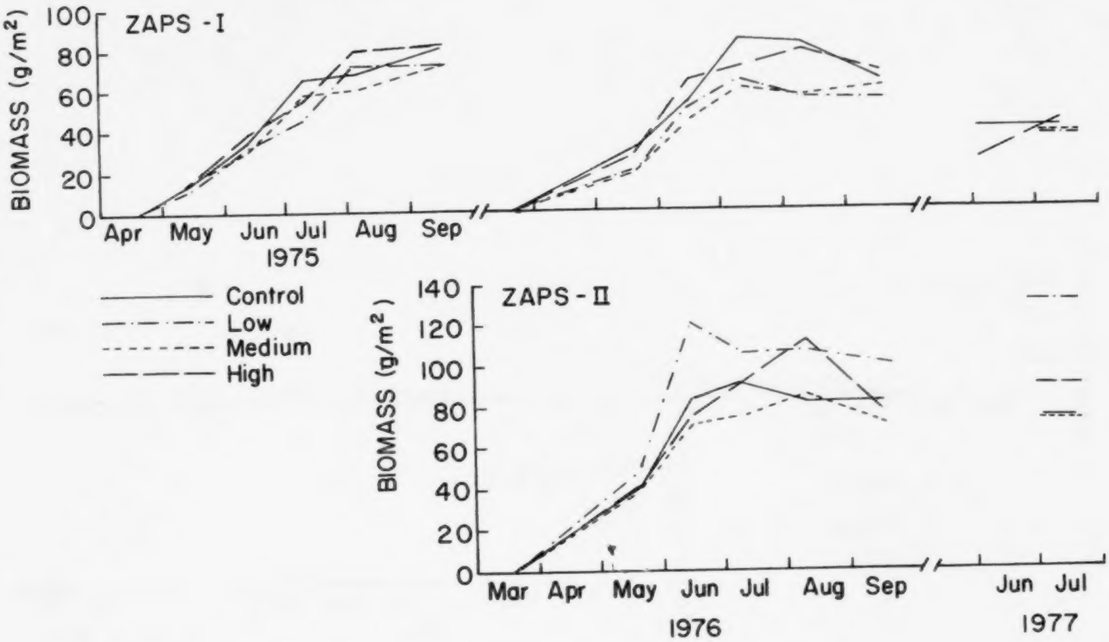


Figure 13.6. Seasonal change in current years production for western wheatgrass, ZAPS I and II, 1975-1977.

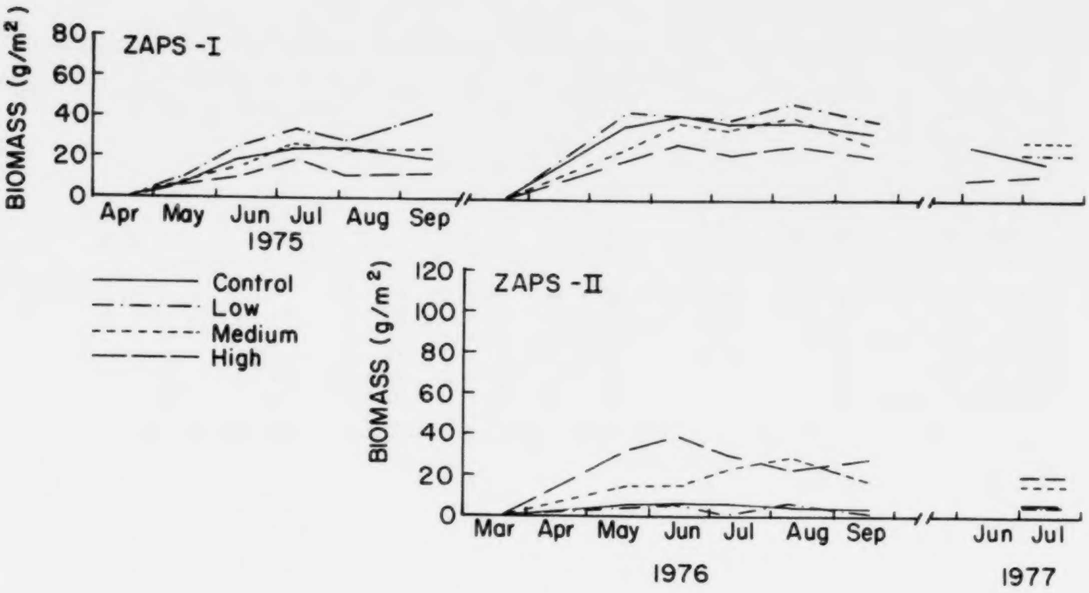


Figure 13.7. Seasonal change in current years production for prairie June grass, ZAPS I and II, 1975-1977.

basis (ash-free organic weight) and expect to be able to detect only gross changes.

Following three and two years of SO_2 stress on the ZAPS I and ZAPS II sites, respectively, we were unable to detect any consistent treatment effect on belowground plant biomass dynamics (Tables 13.2, 13.3, and 13.4). Rhizome biomass appears to have been reduced by three years of exposure of the community to SO_2 (ZAPS I, 1977), but we are reluctant to accept this as significant until we can confirm it by 1978 sampling.

In an attempt to discern the dynamics of the living portion of the rhizome compartment, we measured nonstructural carbohydrate concentrations in samples of western wheatgrass rhizomes throughout the 1976 season (Table 13.5). Again, SO_2 treatment effects on the most labile portion of the live rhizome were not detected. At this time we cannot conclude that the seasonal dynamics of the living portion of belowground live plant biomass are or are not altered by exposure of the aboveground organs to SO_2. We can only conclude that the short-term effects are either nonexistent or not of sufficient magnitude to detect with our sampling procedures.

PHENOLOGY

Phenological development of the major plant species was recorded, by treatment, for the ZAPS study sites from 1975 through 1977. The 1975 results

TABLE 13.2. STANDING CROP OF PLANT CROWNS*

Date	ZAPS I				ZAPS II			
	Control	Low	Medium	High	Control	Low	Medium	High
19 April 1975	64 ± 10	74 ± 9	83 ± 29	70 ± 12				
31 May 1975	35 ± 4	79 ± 13	56 ± 8	46 ± 6				
15 June 1975	70 ± 9	42 ± 5	56 ± 8	37 ± 7				
13 July 1975	53 ± 8	55 ± 15	43 ± 9	33 ± 7				
7 August 1975	71 ± 14	48 ± 9	48 ± 8	47 ± 8				
17 September 1975	77 ± 12	57 ± 10	47 ± 6	29 ± 4				
21 March 1976	68 ± 8	80 ± 11	80 ± 8	74 ± 9	61 ± 8	57 ± 5	55 ± 6	61 ± 8
20 May 1976	46 ± 7	59 ± 8	71 ± 10	54 ± 8	41 ± 5	44 ± 7	43 ± 6	54 ± 7
15 June 1976	94 ± 9	73 ± 9	78 ± 10	74 ± 9	74 ± 7	74 ± 9	77 ± 7	75 ± 9
10 July 1976	63 ± 10	64 ± 9	61 ± 8	63 ± 6	69 ± 9	83 ± 7	68 ± 10	81 ± 7
9 August 1976	54 ± 7	70 ± 10	69 ± 13	67 ± 10	56 ± 7	64 ± 7	79 ± 7	64 ± 7
19 September 1976	80 ± 9	76 ± 9	98 ± 8	81 ± 8	83 ± 8	98 ± 9	85 ± 9	104 ± 11

*$\bar{X}$ ± SE, g · m^{-2}, ash-free

TABLE 13.3. STANDING CROP OF RHIZOMES*

Date	ZAPS I				ZAPS II			
	Control	Low	Medium	High	Control	Low	Medium	High
19 April 1975	36 ± 6	15 ± 2	28 ± 7	29 ± 4				
31 May 1975	22 ± 4	13 ± 3	23 ± 5	22 ± 3				
15 June 1975	39 ± 5	21 ± 5	22 ± 4	27 ± 6				
13 July 1975	26 ± 4	32 ± 9	24 ± 3	24 ± 5				
7 August 1975	27 ± 4	19 ± 4	26 ± 4	19 ± 3				
17 September 1975	33 ± 4	24 ± 6	30 ± 6	26 ± 2				
21 March 1976	24 ± 3	19 ± 3	27 ± 4	25 ±	31 ± 3	23 ± 2	30 ± 6	25 ± 3
20 May 1976	44 ± 5	32 ± 5	30 ± 4	30 ± 3	18 ± 2	23 ± 2	28 ± 5	29 ± 4
15 June 1976	28 ± 2	21 ± 2	22 ± 3	25 ± 2	18 ± 2	29 ± 3	31 ± 5	29 ± 3
10 July 1976	50 ± 5	31 ± 3	34 ± 5	23 ± 2	25 ± 2	36 ± 3	24 ± 3	25 ± 3
9 August 1976	41 ± 3	27 ± 4	25 ± 3	31 ± 5	26 ± 3	33 ± 4	31 ± 5	28 ± 5
19 September 1976	47 ± 6	38 ± 4	34 ± 5	38 ± 5	27 ± 3	39 ± 3	26 ± 4	32 ± 4
12 July 1977	62 ± 8	37 ± 5	43 ± 7	26 ± 4	35 ± 4	38 ± 5	40 ± 8	36 ± 5

*$\bar{X}$ ± SE, g · m^{-2}, ash-free, 0-10 cm depth

have been reported in Dodd *et al.* (1978). The 1976 and 1977 results are reported in Appendix Tables 13.1 and 13.2.

In 1975 and 1976 we used a 14-stage phenophase classification scheme and Dr. John Taylor, another investigator on the Colstrip coal-fired power plant project, used a 16-stage scheme. To standardize the phenology studies conducted by different investigators in the Colstrip program, Dr. Taylor met with our group in December 1976 and we developed a common 12-stage classification scheme. This scheme and its relationship to the previous schemes is shown in Table 13.6. Phenology data presented in Appendix Tables 13.1 and

TABLE 13.4. STANDING CROP OF ROOTS[*]

Date	ZAPS I Control	ZAPS I Low	ZAPS I Medium	ZAPS I High	ZAPS II Control	ZAPS II Low	ZAPS II Medium	ZAPS II High
19 April 1975	714 ± 69	573 ± 42	697 ± 88	769 ± 59				
31 May 1975	564 ± 36	461 ± 35	487 ± 47	614 ± 29				
15 June 1975	673 ± 39	512 ± 51	539 ± 40	498 ± 36				
13 July 1975	490 ± 60	652 ± 75	655 ± 54	625 ± 35				
7 August 1975	523 ± 51	479 ± 20	527 ± 39	482 ± 48				
17 September 1975	618 ± 46	509 ± 39	531 ± 26	520 ± 35				
21 March 1976	597± 40	605± 49	605± 26	596± 26	599 ± 22	553 ± 22	550 ± 26	525 ± 23
20 May 1976	565± 38	489± 27	531± 23	505± 19	477 ± 25	510 ± 18	624 ± 36	613 ± 21
15 June 1976	651± 32	547± 23	504± 25	538± 25	554 ± 25	635 ± 24	685 ± 35	601 ± 21
10 July 1976	506± 26	506± 32	504± 31	552± 23	624 ± 28	721 ± 30	668 ± 29	649 ± 38
9 August 1976	506± 28	565± 57	530± 37	501± 26	452 ± 25	555 ± 48	588 ± 37	572 ± 33
19 September 1976	485± 30	500± 24	548± 25	513± 28	534 ± 36	600 ± 27	570 ± 39	625 ± 22
12 July 1977	492± 30	497± 35	505± 25	558± 22	519 ± 29	651 ± 42	617 ± 39	519 ± 28

[*] $\bar{X}$ ± SE, g · m^{-2}, ash-free, 0-1 cm depth

TABLE 13.5. PERCENT TOTAL AVAILABLE CARBOHYDRATE CONCENTRATION IN RHIZOMES OF WESTERN WHEATGRASS

Date	Control Rep. 1	Control Rep. 2	Low Rep. 1	Low Rep. 2	Medium Rep. 1	Medium Rep. 2	High Rep. 1	High Rep. 2
				ZAPS I				
26 March			11	11	11	11	7	10
26 May	9	8	10	12	11	11	13	8
24 June	9	9	12	12	11	9	9	15
19 July	16	10	17	13	12	15	13	12
16 August	24	21	19	17	24	20	16	14
20 September	10	14	12	17	11	14	11	10
Average	14	12	13	13	13	13	11	11
				ZAPS II				
26 March					11	7	8	9
26 May		7	9	9	8	7	11	6
24 June	5	7	5	7	12	12	6	7
19 July	14	12	11	15	21	13	9	12
16 August	20	27	18	19	27	17	20	21
20 September	16	19	19	25	23	19	18	23
Average	14	14	12	15	17	12	12	13

13.2 are according to the 12-stage scheme. The 1976 data were collected using the 14-stage scheme and converted to the 12-stage scheme.

Rates of phenological progression do not appear to have been greatly altered by the SO_2 treatments for any of the species observed in either ZAPS experiment. However, there is some indication that the rate of phenological

TABLE 13.6. COMPARISON OF PHENOLOGY SCHEMES

Codes			
Pre-1977		CSU-MSU	
MSU	CSU	1977	Growth stage
4, 14	2	1	First growth
4, 5	3	2	First leaves fully expanded
5	4, 5	3	Active vegetative growth
5	6	4	Vegetative growth mostly complete
6	7	5	Root stage, first floral buds
6	8	6	Exsertion of grass inflorescences, earliest flowers
7, 8	9	7	Reproductive culms fully extended
8, 9	10	8	Anthesis, full flowering
9	11	9	Fruit developing
10	12	10	Fruit ripe
11, 12	13	11	Dehiscence
2, 13, 15, 16	14, 1	12	Vegetative maturity, summer or winter dormancy, leaf drop, annuals dead

progression between anthesis (stage 8) and dehiscence (stage 11) may have been delayed by the SO_2 treatments for *Agropyron smithii* in 1977 on both ZAPS sites. This may or may not be a significant effect.

Although major changes in phenological progression did not result from exposure to chronic levels of SO_2, the phenology data indicate key differences among species and among years. Western wheatgrass and prairie June grass, the two most abundant species on the sites, differ considerably in their phenological development (Figure 13.8). Although both species are cool season species and initiate active vegetative growth at about the same time (April), prairie June grass completes vegetative growth and attains sexual maturity (anthesis) 2 to 4 weeks earlier than does western wheatgrass.

Active vegetative growth (stage 3) was initiated later in 1977 than in 1976 for many species. Western wheatgrass was about 2 weeks later and prairie June grass was about a month later.

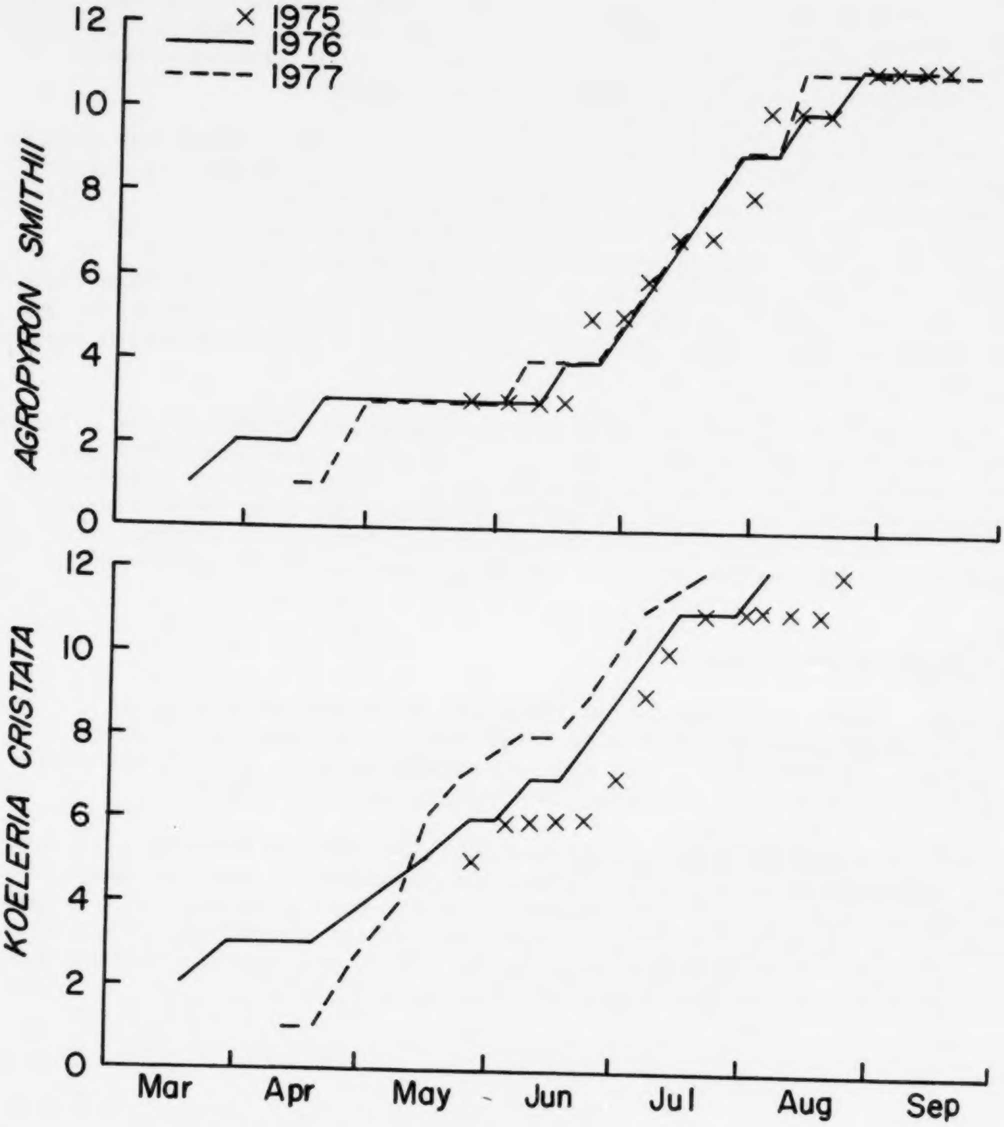


Figure 13.8. Phenological progression of *Agropyron smithii* and *Koeleria cristata* for ZAPS I control treatment, 1975-1977 (see Table 13.6 for phenology code).

EFFECTS OF CONTROLLED LEVELS OF SO_2 ON THE NUTRIENT QUALITY OF WESTERN WHEATGRASS AND PRAIRIE JUNE GRASS*

The principal economic value of northern Great Plains grasslands is directly related to their capacity to produce quality grazing forage. The range livestock industry is the single most important economic use of these grasslands. Alteration of forage quality by air pollutants as a result of energy development would adversely affect this industry.

Sulfur is essential in ruminant diets since it is an important constituent in protein synthesis. Rumen microorganisms require elemental sulfur for synthesis of microbial protein (Loosli *et al.*, 1949; Thomas *et al.*, 1951; Starks *et al.*, 1954). Cellulose digestion, in vitro, has been shown to increase with addition of sulfate to prepared rations (Hunt *et al.*, 1954; Martin *et al.*, 1964; Barton *et al.*, 1971). However, sulfur has been reported to be toxic to rumen microorganisms in vitro at a concentration of 100 ppm from sodium sulfate (Hubbert *et al.*, 1955) and at 30 ppm from sodium sulfite (Trenkle *et al.*, 1958).

The objective of the research reported here was to examine the effects of SO_2 fumigation on the nutritive quality of two of the most important forage species in the northern Great Plains grasslands, western wheatgrass (*Agropyron smithii* Rydb.) and prairie June grass (*Koeleria cristata* (L.) Pers.). Nutritive quality was assessed by examining crude protein, cell-wall constituents, ash, sulfur content, and digestibility of dry matter in vitro.

Field Sampling Procedures

Plant tissue for chemical analyses was collected on four dates for two years. Sample dates for 1975 were 16-17 May, 12-15 June, 10-13 July, and 4-7 August. In 1976 Site I was always sampled prior to Site II and sample dates were 17-24 May, 15-23 June, 10-17 July, and 9-16 August.

Harvest quadrats were located randomly in each replicate on each sample date. All vegetation was then clipped and separated by species into current live, recent dead (current year's dead), or old dead (previous year's dead). Subsamples of current live plant tissue for western wheatgrass were chemically analyzed for each sample date at both sites and years; prairie June grass was analyzed for all sample dates at Site I, 1976.

---

[*] Major portion of this section taken from a manuscript authored by C. C. Schwartz, W. K. Lauenroth, R. K. Heitschmidt, and J. L. Dodd. Effects of controlled levels of sulphur dioxide on the nutrient quality of western wheatgrass, *Journal of Applied Ecology* 15(3):869-874, 1978.

Laboratory Techniques

Crude protein (Kjeldahl N × 6.25) and ash were determined by procedures in A.O.A.C. (1965). Cell-wall constituents (CWC) were determined by procedures outlined by Van Soest and Wine (1967). Total sulfur concentrations were determined with a Leco Induction Furnace (Laboratory Equipment Corp., St. Joseph, Michigan).

Digestion of dry matter (DMD) was determined in vitro using techniques described by Tilley and Terry (1963) and Pearson (1970). Samples were ground in a Wiley mill to pass through a 0.5-mm screen. Inoculum was prepared by adding one part of strained rumen fluid to four parts of prewarmed (38.5°C) standard buffer solution (McDougall, 1948; Table 13.11) saturated with CO_2. Inocula were obtained from a fistulated bovine cow maintained on grass hay. Duplicate 250-mg samples were tested for each replicate on each date.

Data were analyzed with a repeated measure analysis of variance (ANOVA) repeating across time (Winer, 1971). ANOVA tests were performed on data for western wheatgrass and for prairie June grass. Throughout the text, reference is made to year-site effects for western wheatgrass. The year-site source of variation contrasts Site I-1975, Site I-1976, and Site II-1976 and therefore includes both differences between 1975 and 1976 and between Sites I and II. Tukey's Q values were utilized to identify significant differences between means (Snedecor and Cochran, 1967). Significant differences referred to in the text are at the $P = 0.01$ level for analysis of variance and $P = 0.05$ for Q values.

Appendix Tables 13.3 and 13.4 include results of chemical analysis of western wheatgrass and prairie June grass plant tissue collected from ZAPS I and II plots for 1975 and 1976. Similar analyses have been made of plant tissue of other species collected on the fumigation sites and results are on file at the Natural Resource Ecology Laboratory, Colorado State University, Fort Collins.

Results and Discussion

Sulfur

Analysis of sulfur data for western wheatgrass indicated significant year-site, treatment, date, treatment × date, and treatment × year-site effects (Table 13.7). There were also significant treatment, date, and treatment × date ($P < 0.02$) effects for prairie June grass on Site I-1976.

Sulfur concentrations of plants in the high and medium treatments were significantly greater than plants in the control for all dates except June when no difference was noted between plants in the control and medium plots (Figure 13.9). Sulfur concentration of plants in the low treatment was not significantly different from that of plants in the control. Comparisons between sulfur accumulation rates in tissue were greatest in the high treatment, (0.0022% per day) followed by the medium (0.0015% per day) and low (0.0002% per day) treatments. Concentrations in the control plants did not

TABLE 13.7. PERCENT SULFUR CONCENTRATION OF WESTERN WHEATGRASS AND PRAIRIE JUNE GRASS TISSUE

Site and treatment	Year	Dates				Treatment mean
		May	June	July	August	
		Western wheatgrass				
Site I	1975					
Control			0.11	0.10	0.12	0.11^{*}
Low		0.13	0.14	0.14	0.13	0.14^{*}
Medium		0.14	0.21	0.24	0.26	0.21^{*}
High		0.13	0.23	0.28	0.33	$0.24^{\dagger}$
Date mean		0.13^{*}	$0.18^{\dagger}$	$0.19^{\dagger}$	$0.21^{\dagger}$	
Site I	1976					
Control		0.07	0.09	0.09	0.08	0.09^{*}
Low		0.11	0.13	0.13	0.15	$0.13^{\dagger}$
Medium		0.16	0.21	0.22	0.29	$0.22^{\ddagger}$
High		0.23	0.24	0.32	0.41	$0.30^{\S}$
Date mean		0.15^{*}	0.17^{*}	0.19^{*}	$0.24^{\dagger}$	
Site II	1976					
Control		0.13	0.11	0.10	0.10	0.11^{*}
Low		0.14	0.12	0.17	0.14	0.14^{*}
Medium		0.16	0.17	0.27	0.28	$0.22^{\dagger}$
High		0.32	0.35	0.43	0.48	$0.40^{\ddagger}$
Date mean		0.19^{*}	0.19^{*}	$0.24^{\dagger}$	$0.25^{\dagger}$	
		Prairie June grass				
Site I	1976					
Control		0.09	0.09	0.08	0.07	0.08^{*}
Low		0.08	0.13	0.16	0.18	$0.14^{\dagger}$
Medium		0.18	0.21	0.21	0.25	$0.21^{\ddagger}$
High		0.21	0.24	0.27	0.38	$0.28^{\S}$
Date mean		0.14^{*}	0.17^{*}	0.16^{*}	$0.22^{\dagger}$	

$^{*\ \dagger\ \ddagger\ \S}$/Any two means not sharing common superscripts within a row or column within a year-site are significantly different ($P < 0.05$).

change with time. Similar trends in sulfur concentration by treatment and date were apparent for prairie June grass.

Comparisons of year-site differences indicated sulfur levels were significantly higher ($P < 0.01$) on Site II-1976 (0.22%) than on Site I-1976 (0.18%) and Site I-1975 (0.17%). The year-site × treatment interaction resulted from different sulfur levels in the high treatment at all site-years (Figure 13.10).

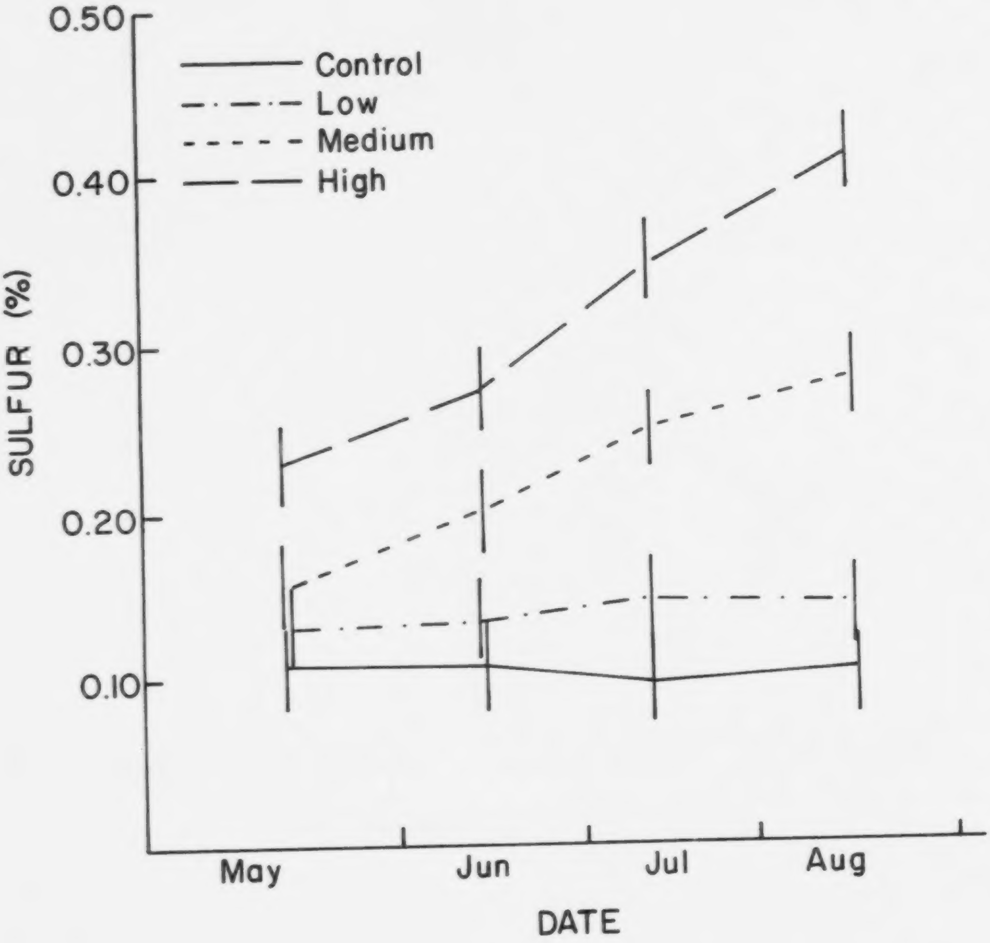


Figure 13.9. Change in percent sulfur in western wheatgrass for four dates on four treatments: control (0 pphm SO_2), low (2 pphm SO_2), medium (5 pphm SO_2), and high (10 pphm SO_2). Plotted values are means from Sites I-1975, I-1976, and II-1976. Treatment means with overlapping vertical lines are not significantly different at P = 0.1 (Q = 0.05).

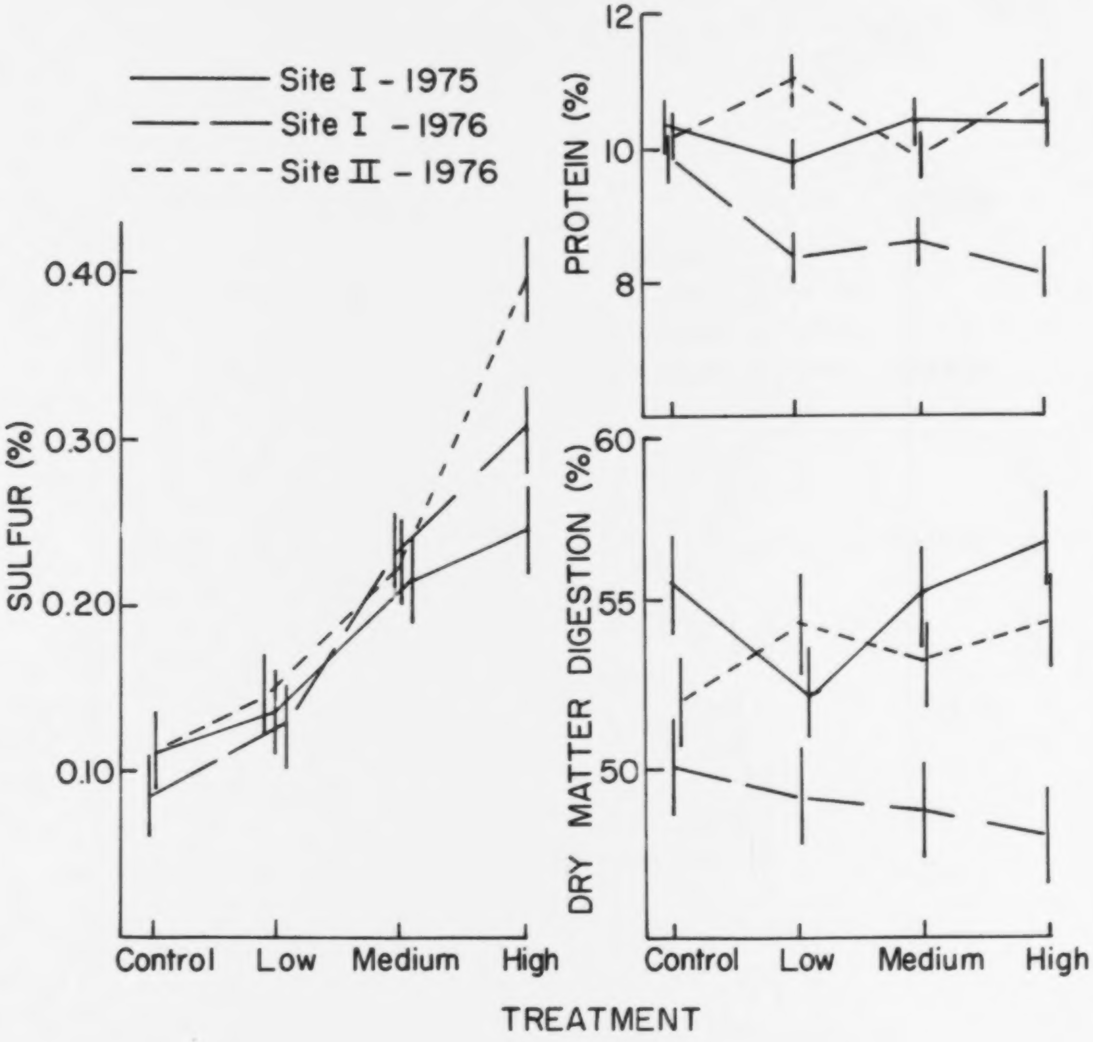


Figure 13.10. Changes in sulfur (%), protein (%), and digestion of dry matter in vitro (%) in western wheatgrass for four treatments (control, 0 pphm; low, 2 pphm; medium, 5 pphm; high, 10 pphm) for three site years. Site year means within a fumigation level with overlapping vertical lines are not significantly different at P = 0.05 (Q = 0.05) for sulfur, at P = 0.05 (Q = 0.08) for protein, and at P = 0.05 (Q = 2.9) for dry matter digestion.

Comparisons between Site I-1975 and Site I-1976 revealed sulfur concentrations were significantly higher in 1976 than in 1975. This was probably because of the increased period of fumigation in 1976 (196 days) compared to that in 1975 (151 days).

Ash

Ash concentrations in western wheatgrass tissue paralleled sulfur concentrations with higher levels of ash occurring with higher concentrations of sulfur (Table 13.8). However, the magnitude of fluctuations in ash concentrations across dates was greater than that of fluctuations in total sulfur. The significant year-site, year-site × treatment, and date × year-site effects were similar to results reported for sulfur analysis.

Total ash concentrations were greater on Site II in 1976 (8.5%) than on Site I (7.5%). This was probably a result of differences in sulfur concentrations. Ash levels in plant tissue were determined by ashing plants at 500°C. At this temperature, sulfate salts remain as ash residue. Sulfur dioxide from fumigation is converted to sulfate salts in plant tissues (Thomas *et al.*, 1951). If this conversion is considered stochiometrically by converting a sulfur molecule (molecular weight: 32) to a sulfate salt molecule containing two calcium ions (molecular weight: $2 \times 20 = 40$), the resulting increase in molecular weight is approximately four times greater for the sulfate salt than for the sulfur molecule. Differences in sulfur concentration from control to high treatments varied between 0.13% to 0.29% for both species and site. Likewise, differences in ash concentrations varied from 0.6% to 2.2% between the control and the high treatment. The four-fold increase in weight from sulfur to sulfate salt would therefore account for an increase of 0.6% to 1.0% in ash weight if all sulfur molecules were in the form of calcium sulfate. If the cation involved was potassium, the increase in molecular weight from sulfur to potassium sulfate would be 5.4 times greater and would account for a 0.9% to 1.3% increase in ash weight. It was probable that the major increase in ash content across treatments was a direct result of increased sulfate salt concentrations rather than a change in plant ash as a result of fumigation. Ziegler (1975) stated that in spruce needles, SO_2 caused an increase in calcium, magnesium, silicon, and, during springtime, potassium.

Crude Protein

Concentrations of crude protein in western wheatgrass and prairie June grass varied significantly between sampling dates with highest values occurring in May and lowest in August (Table 13.9). This relationship obtained regardless of sulfur treatment.

Comparisons between year-sites for western wheatgrass indicated significantly lower concentrations of crude protein for two years (Site I-1976, 8.8%) of fumigation compared to either value for one year (Site I-1975, 10.6%; Site II-1976, 10.2%) of treatment. The significant year-site × treatment interaction (Figure 13.10) illustrated the source of lower crude

TABLE 13.8. PERCENT ASH CONTENT OF WESTERN WHEATGRASS AND PRAIRIE JUNE GRASS TISSUE

Site and treatment	Year	Dates May	 June	 July	 August	Treatment mean
			Western wheatgrass			
Site I	1975					
Control		--	7.7	6.8	7.6	7.4*
Low		8.5	7.8	6.4	7.2	7.5*
Medium		8.4	8.1	7.7	7.8	8.0†
High		8.3	8.1	8.3	8.6	8.3†
Site I	1976					
Control		6.9	6.5	6.6	6.9	6.7*
Low		7.1	7.4	6.9	7.9	7.3†
Medium		7.2	7.1	7.0	8.2	7.4†
High		8.2	7.9	8.3	9.5	8.5‡
Site II	1976					
Control		9.0	7.6	8.6	8.3	8.4†
Low		8.3	6.4	8.4	7.4	7.6*
Medium		9.6	8.3	9.0	9.0	8.9‡
High		9.4	8.2	9.2	9.3	9.0‡
			Prairie June grass			
Site I	1976					
Control		8.5	10.2	10.0	9.9	9.6*
Low		9.0	9.6	10.1	10.4	9.9*†
Medium		10.0	9.9	11.6	10.6	10.4†‡
High		10.5	11.8	12.4	12.9	11.9§

$^{*\,\dagger\,\ddagger\,\S}$/Any two means not sharing common superscripts within a column and year-site are significantly different ($P < 0.05$).

TABLE 13.9. PERCENT PROTEIN (KJELDAHL N × 6.25) CONTENT OF WESTERN WHEATGRASS AND PRAIRIE JUNE GRASS TISSUE

Site and treatment	Year	Dates May	 June	 July	 August	Treatment mean
		Western wheatgrass				
Site I	1975					
Control		15.6	10.6	8.1	6.9	10.4^{*}
Low		15.0	10.6	7.5	6.2	9.8^{*}
Medium		15.6	10.6	8.1	6.9	10.4^{*}
High		16.2	11.2	7.5	6.2	10.4^{*}
Site I	1976					
Control		14.4	9.4	9.4	6.1	9.8^{*}
Low		13.8	8.8	6.9	5.0	$8.4^{\dagger\ddagger}$
Medium		12.5	9.4	6.9	5.8	$8.6^{*\ddagger}$
High		11.9	8.1	6.9	5.6	$8.2^{\dagger\ddagger}$
Site II	1976					
Control		14.4	11.9	8.8	6.2	10.3^{*}
Low		15.0	11.9	10.0	7.5	11.1^{*}
Medium		14.4	10.0	8.1	6.2	9.8^{*}
High		15.6	11.9	9.4	7.5	11.1^{*}
		Prairie June grass				
Site I	1976					
Control		11.2	8.2	7.1	7.6	8.5^{*}
Low		10.4	8.3	7.1	6.7	8.1^{*}
Medium		10.6	8.2	7.1	7.3	8.4^{*}
High		10.6	8.4	6.8	7.4	8.1^{*}

$^{*\,\dagger\,\ddagger}$/Any two means not sharing common superscripts within a column and year-site are significantly different ($P < 0.05$) (Kjeldahl N × 6.25).

protein concentrations. Crude protein levels of plant tissue from fumigated plots were significantly lower than those from the control at Site I-1976. Plots fumigated for one year (Site I-1975, Site II-1976) were not different from each other or their controls (Figure 13.10). Protein concentrations on control plots were not significantly different between the three year-sites. It appeared that crude protein levels were lower on treated plots compared to the control at Site I-1976.

The significant reduction of crude protein in western wheatgrass in treated plots compared to the control at Site I-1976 was attributed to sulfur dioxide fumigation. Heitschmidt (1977) reported increased leaf senescence of western wheatgrass during both years of fumigation at Site I. Since crude protein content was clearly related to plant age, this alone would provide an explanation for the reduction, except that increased senescence occurred in both 1975 and 1976; significant reduction in concentration of crude protein was detected only in 1976. Although decreases in forage quality as a result of sulfur dioxide fumigation have been alluded to by other workers (Guderian, 1977), to our knowledge reduced crude protein in grasses has not previously been reported.

Most proteins in plant tissue are enzymatic in structure. Bailey and Cole (1959), Zelitch (1960), and Cecil and Wake (1962) have demonstrated that sulfur dioxide can inactivate or inhibit many enzyme systems; it is possible that these same mechanisms are responsible for reductions in total plant proteins.

Cell Wall Constituents

Cell-wall constituents (CWC) varied significantly ($P < 0.01$) between dates (Table 13.10) for both grass species. There was also a significant ($P < 0.01$) year-site and date × year-site interaction for western wheatgrass. Percentage of CWC was lowest in May samples and increased during other months. Trends in the year-site × treatment interaction indicated that the percent CWC at Site II-1976 was lower than the other two sites during the June sampling period; all other dates and sites were not different. We have no explanation for this difference.

The nonsignificant treatment and treatment × date interaction indicated SO_2 fumigation did not measurably alter the fiber content of plant tissue. Significant effects were hypothesized since increases in leaf senescence (Heitschmidt, 1977) should increase total fiber content. These results verify that the significant reductions in crude protein resulting from two years of treatment were not simply the results of differences in proportions of live and dead tissue among treatments.

Dry Matter Digestion

Digestion of dry matter (DMD) in vitro (Table 13.11) varied significantly across dates for both grass species. Plant tissue was highly

TABLE 13.10. PERCENT CELL-WALL CONSTITUENTS OF WESTERN WHEATGRASS AND PRAIRIE JUNE GRASS TISSUE

Site and treatment	Year	Dates				Treatment mean
		May	June	July	August	
		Western wheatgrass				
Site I	1975					
Control		65.8	71.6	69.3	65.7	68.1
Low		64.8	74.6	69.1	67.1	68.9
Medium		63.0	74.1	70.1	67.7	68.7
High		60.4	70.5	69.6	69.3	67.5
Site I	1976					
Control		64.9	70.9	69.3	68.4	68.4
Low		68.1	71.5	68.0	65.9	68.4
Medium		66.5	70.0	69.3	67.1	68.2
High		63.0	67.6	66.4	65.7	65.7
Site II	1976					
Control		61.5	61.8	64.0	64.8	63.0
Low		60.7	65.0	64.9	65.0	63.9
Medium		60.8	66.1	65.8	64.9	64.4
High		62.5	67.3	65.9	63.4	60.6
		Prairie June grass				
Site I	1976					
Control		60.0	68.1	66.0	61.4	63.9
Low		62.8	67.1	64.7	60.9	63.9
Medium		62.9	66.5	65.6	59.5	63.4
High		64.1	65.4	67.7	60.6	64.6

TABLE 13.11. PERCENT DRY MATTER DIGESTION OF WESTERN WHEATGRASS AND PRAIRIE JUNE GRASS TISSUE

Site and treatment	Year	Dates				Treatment mean
		May	June	July	August	
		Western wheatgrass				
Site I	1975					
Control		72.0	53.8	51.5	45.5	55.6
Low		64.3	54.3	47.4	43.6	52.4
Medium		70.0	55.1	50.4	44.8	55.2
High		69.0	59.7	51.7	46.4	56.8
Site I	1976					
Control		60.5	56.1	48.6	42.9	50.0
Low		66.9	54.4	50.8	45.1	49.2
Medium		66.7	52.7	50.2	43.3	48.8
High		69.2	54.6	47.9	46.5	48.0
Site II	1976					
Control		62.8	49.4	46.2	41.8	52.0
Low		62.4	48.6	44.4	41.8	54.4
Medium		60.6	50.4	44.5	40.8	53.2
High		58.2	50.6	45.0	38.7	54.4
		Prairie June grass				
Site I	1976					
Control		64.0	46.8	46.4	50.4	51.9
Low		61.9	50.2	47.9	45.0	51.2
Medium		64.7	54.6	47.6	52.4	54.8
High		62.0	54.8	46.0	50.2	53.2

digestible in May when plants were more succulent and steadily declined thereafter. This seasonal change was consistent regardless of treatment.

Digestion of dry matter was significantly higher on treatments fumigated for one year (Site I-1975, 55.2; Site II-1976, 53.6) compared to two years of fumigation (Site I-1976, 49.2). The year-site × treatment interaction was significant ($P < 0.02$) and the trend (Figure 13.10) was similar to changes in crude protein concentration. Reduced DMD of western wheatgrass tissue when fumigated for two years was probably the result of increased leaf senescence and reduced protein concentrations because of SO_2 fumigation. Differences in DMD on control plots at Site I in 1975 and 1976 were attributed to differences in growth stage and plant development.

Although there was a reduction in DMD with two years of SO_2 fumigation, coefficients of digestion for treatment levels were not significantly lower than the control at Site I-1976; total protein levels were significantly lower. This nonsignificant effect could have been a result of increased microbial activity caused by higher sulfur levels on treated plant tissues. As discussed earlier, some investigators have shown increased fiber digestion with increased levels of sulfur. This potential sulfur-microbe interaction rendered it difficult to determine effects of SO_2 fumigation on digestion of dry matter. It could also indicate that changes in nutrient quality, particularly protein, are not a direct result of increased leaf senescence. As a result, additional studies, including in vivo digestion trials, are pertinent to the understanding of this problem of sulfur fumigation on forage digestibility.

Conclusions

(1) Sulfur content of plant tissue increased with increasing SO_2 treatment.

(2) Ash concentrations increase in a parallel manner with sulfur concentrations.

(3) Crude protein in western wheatgrass was decreased significantly by two years of treatment.

(4) Cell wall constituents were not altered by SO_2 treatment.

(5) Dry-matter digestibility was reduced by two years of treatment and seemed to parallel crude protein.

Summary

Effects of low-level sulfur dioxide (SO_2) fumigation on nutrient quality of western wheatgrass and prairie June grass were investigated. Analyses indicated significantly higher levels of sulfur on fumigated vs. control plots. Accumulation rates of sulfur in tissues were greatest in the high treatment (0.0022% per day) followed by the medium (0.0015% per day) and low

(0.0002% per day) treatments. Concentrations of sulfur in control plants did not change with time. Ash concentrations paralleled sulfur concentrations and major increases in ash content of plant tissue were attributed to the increased levels of sulfur. Crude protein levels varied seasonally, regardless of fumigation level, with the highest concentrations in May and the lowest in August. There were significantly lower levels of crude protein on treatment plots fumigated for 2 years compared to controls. This significant reduction was attributed to SO_2 fumigation. Cell-wall constituent analysis indicated SO_2 fumigation did not measurably alter the fiber content of plant tissue. Digestion of dry matter was significantly higher on treatments fumigated for one year than those fumigated for two years. In general, digestion of dry matter paralleled crude protein concentrations.

EFFECTS OF CONTROLLED LEVELS OF SO_2 ON GROWTH AND SENESCENCE OF WESTERN WHEATGRASS*

An important component of our investigations of the effects of sulfur dioxide on primary production and biomass dynamics has been concerned with the growth and development of western wheatgrass (*Agropyron smithii*). In this section we report these results in two parts. The first presents information from the 1975 and 1976 growing seasons.* The second section contains data collected during the 1977 growing season and is presented without statistically based interpretations.

Growth and Senescence (1975-1976)

Few studies of the effects of long- or short-term exposures of grasses or grasslands to low-level SO_2 concentrations have been conducted. Bell and Clough (1973) exposed ryegrass (*Lolium perenne* L.) plants to a 12-pphm concentration of SO_2 for nine weeks and found a 46% reduction in dry-weight yield. Similarly, they reported a 52% reduction in dry weight when ryegrass plants were exposed to 6.7 ppm SO_2 for 26 weeks. Bleasdale (1973) found similar responses in ryegrass exposed to low levels of "coal-smoke." In both of these studies the reduction in growth occurred without detectable visible leaf injury. Katz (1949) found that reductions in yield of alfalfa (*Medicago sativa* L.) induced by SO_2 exposures did not occur without visible injury of the leaves. Other studies utilizing low-level SO_2 exposures have shown at least temporary reductions in photosynthetic rates of plants (which could possibly lead to reduced yields) without measurable leaf injury (Thomas *et al.*, 1950; Daines, 1968; Bennett and Hill, 1973; Siji and Swanson, 1974; Malhorta and Hocking, 1976).

---

*Major portion of this section taken from manuscript authored by R. K. Heitschmidt, W. K. Lauenroth, and J. L. Dodd. Effects of controlled levels of sulphur dioxide on western wheatgrass in a southeastern Montana grassland, *Journal of Applied Ecology* 15(3):859-868, 1978.

The objectives of the present study were to determine the effects of SO_2 on western wheatgrass (*Agropyron smithii* Rydb.), the dominant species of most grasslands of the northern Great Plains. Our specific objectives were to determine the effects of SO_2 on leaf area, leaf surfaces, relative growth rates, net assimilation rates, and leaf area ratios of western wheatgrass. During the June, July, August, and September biomass sample dates in 1975 and the May, June, July, and August biomass sample dates in 1976, 20 western wheatgrass plants were collected randomly from each treatment replicate for leaf area analyses. All leaf blades were then removed and mounted between two pieces of clear adhesive acetate and total and necrotic adaxial surface area of each leaf determined visually, utilizing a clear acetate 0.5-cm^2 grid.

Leaf injury was defined as the percentage of necrotic leaf area in excess of control plants. Missing leaves or portions of missing leaves were considered necrotic. Missing areas were estimated by subtracting the portion present from the average total area for leaves of similar age.

Methods employed in growth analyses were developed following Radford (1967). Biomass of an individual western wheatgrass plant ($mg \cdot plant^{-1}$) was calculated using our biomass estimates and density data collected both in 1975 and 1976 by John Taylor (unpublished). From these data we calculated relative growth rates (RGR), net assimilation rates (NAR), and leaf area ratios (LAR).

Statistical analyses consisted of analysis of variance (ANOVA) with Tukey's Q values utilized to contrast paired means (Snedecor and Cochran, 1967). Throughout the manuscript reference is made to year-site effects which contrast Site I-1975, Site I-1976, and Site II-1976 and therefore includes both differences between years and sites. Leaf area analyses were limited to the months of June, July, and August since statistical analyses of the September 1975 and May 1976 data indicated no significant treatment effects.

Results

Growing Conditions

Mean monthly temperatures reflect a warmer growing season in 1976 than in 1975, particularly during May, August, and September (Figures 13.11 and 13.12). In 1975, the mean temperatures for these months were 11.1°, 20.6°, and 15.5°C, respectively, while in 1976 they were 13.2°, 23.4°, and 19.3°C, respectively. As expected, vapor pressure deficits were highly correlated with temperature, and differences between 1975 and 1976 in vapor pressure deficits were similar to temperature differences.

Long-term precipitation records at Broadus, Montana, indicated May and June were wetter than normal in both 1975 and 1976, July and August were near normal, and September was drier than normal (Section 11). Since Broadus is located approximately 50 km northeast of the sites, the applicability of these data for describing deviations from normal conditions at the sites is

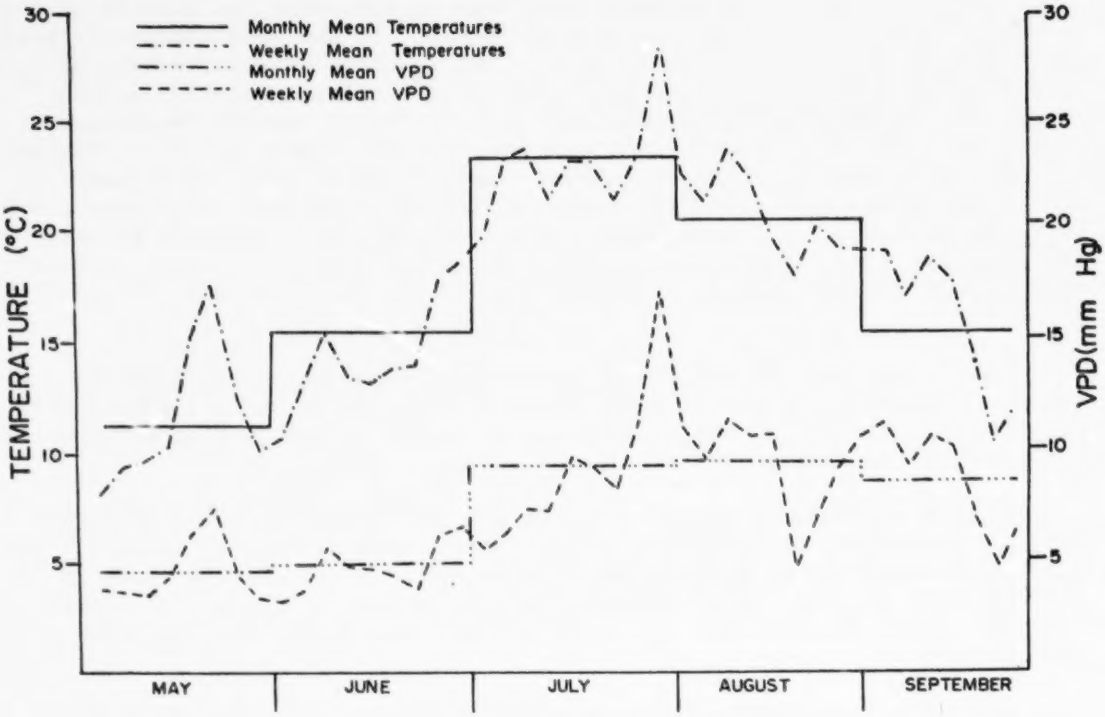


Figure 13.11. Monthly and weekly mean temperatures (°C) and vapor pressure deficits (VPD) (mm Hg) for the 1975 growing season.

limited. However, local ranchers also indicated both years were characterized by above-average spring precipitation.

Total soil water in the 105-cm profile did not vary significantly ($P > 0.10$) between treatments on Site I in 1975 or 1976. However, there were significant ($P < 0.01$) treatment differences on Site II. The low and medium treatment plots averaged a significantly greater quantity of soil water than did the control or high treatment plots. Examination of soil water by 15-cm increments across all sample dates indicated differences in total soil water resulted from minor differences throughout the profile rather than from large differences at any specified depth.

In order to evaluate differences in total soil water among Site I-1975, Site I-1976, and Site II-1976, total soil water of each of the respective control plots was contrasted across eight sample dates. Analysis was limited to control plots since they were sampled more frequently than treatment plots. The ANOVA indicated significant ($P < 0.01$) year-site and date differences and a significant year-site × date interaction. Mean centimeters of soil water for the eight sample dates contrasted were 18.4 cm for Site I-1975, 16.5 cm for Site I-1976, and 12.8 cm for Site II-1976, with all means significantly different. The year-site × date interaction (Figure 13.13)

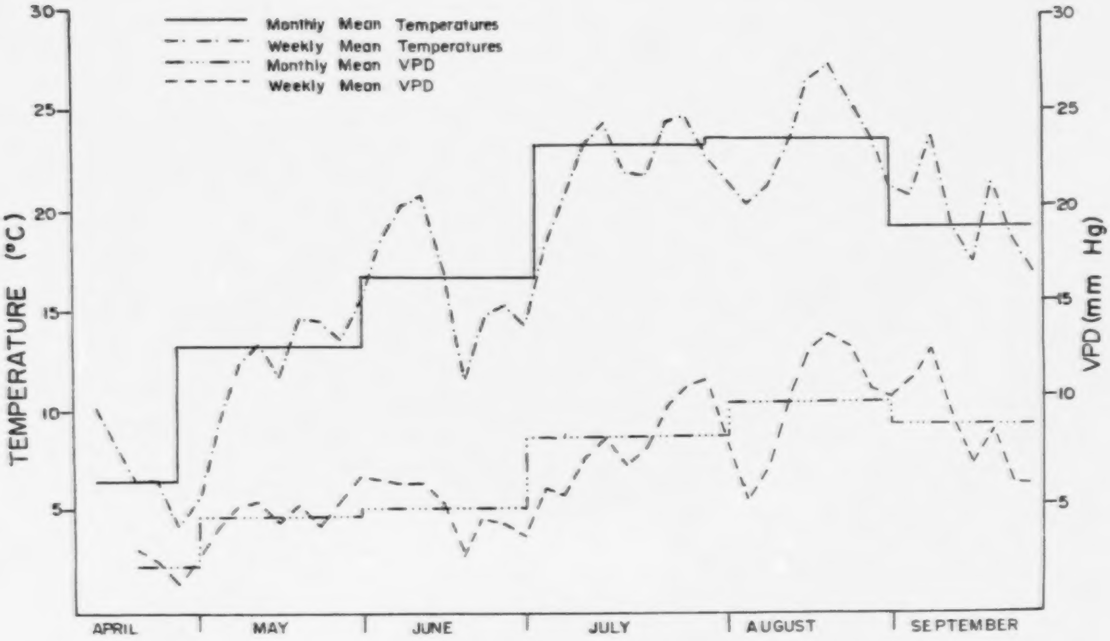


Figure 13.12. Monthly and weekly mean temperatures (°C) and vapor pressure deficits (VPD) (mm Hg) for the 1976 growing season.

suggested differences between treatments were primarily the result of differences during the spring months. Difference between total soil water of the two sites in 1976 was primarily attributed to greater rainfall in May on Site I than on Site II.

Subtle Effects on Leaf Area

The date effect emphasizes the characteristics of the growing season of this region (Table 13.12). Temperatures through the end of June in both 1975 and 1976 were cool and precipitation was abundant. However, near mid-July growing conditions became less favorable as soil water was depleted and average air temperatures increased. These conditions caused a reduction in growth as reflected by the lack of an increase in leaf area from July to August.

Measurements of total leaf area indicate minor differences in growing conditions between 1975 and 1976 and between sites in 1976 (Figure 13.14). Rapid increase in total leaf area from June to July in 1975 was attributed to more favorable soil water conditions than in 1976 (Figure 13.13). Absence of growth from July to August in 1975 was attributed to the very warm temperatures during this time interval (Figure 13.11). Williams (1974)

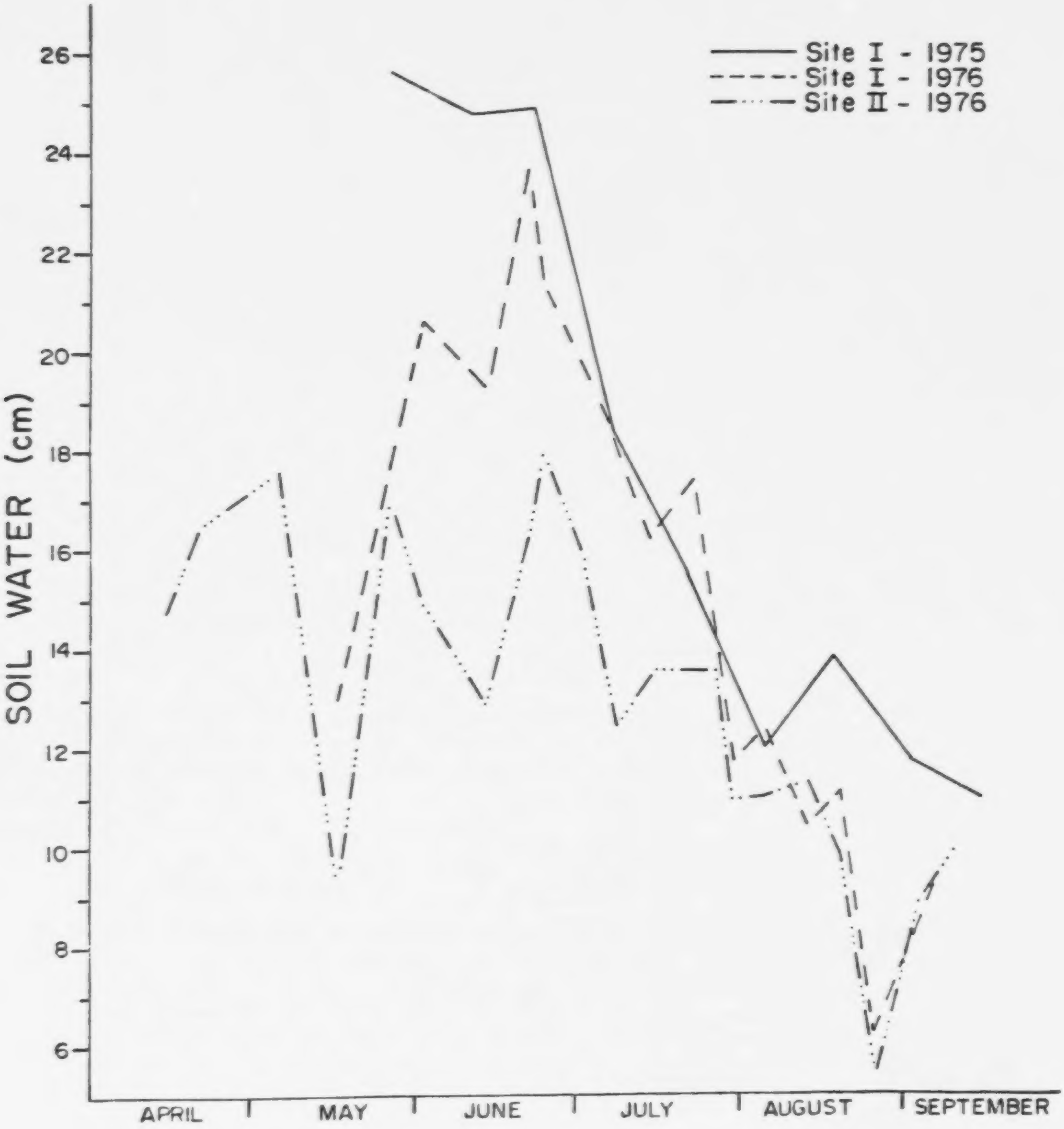


Figure 13.13. Seasonal dynamics of total soil water (cm) to a depth of 105 cm in control plots for 1975 and 1976 growing seasons on Site I and 1976 growing season on Site II.

TABLE 13.12. MEAN VALUE OF WESTERN WHEATGRASS PLANTS FOR VARIOUS MEASURED PARAMETERS[*]

Parameters	Date				
	May	June	July	August	September
Total leaf area (cm^2)	--	26.1_a[†]	35.9_b	36.4_b	--
Mean relative leaf growth rate ($cm^2 \cdot cm^{-2} \cdot week^{-1}$)	--	0.29_a	0.07_b	0.00_c	--
Number of leaves	--	3.6_a	4.9_b	5.2_b	--
Total necrotic leaf area (%)	--	23.6_a	31.4_b	45.4_c	
Necrotic leaf area of four oldest leaves (%)	--	23.1_a	37.8_b	54.9_c	
Biomass (mg)	78.0_a	170.0_b	244.0_c	267.0_d	267.0_d
Mean relative growth rate ($mg \cdot mg^{-1} \cdot week^{-1}$)	0.70_a	0.19_b	0.10_c	0.03_d	0.00_d

[*] Mean values are averages of control, low, medium, and high SO_2 treatments for Site I-1975, Site I-1976, and Site II-1976. Average biomass and mean relative growth rates based only on data from Site I in 1975 and 1976.

[†] Means in a row followed by the same letter are not significantly different at $P = 0.05$.

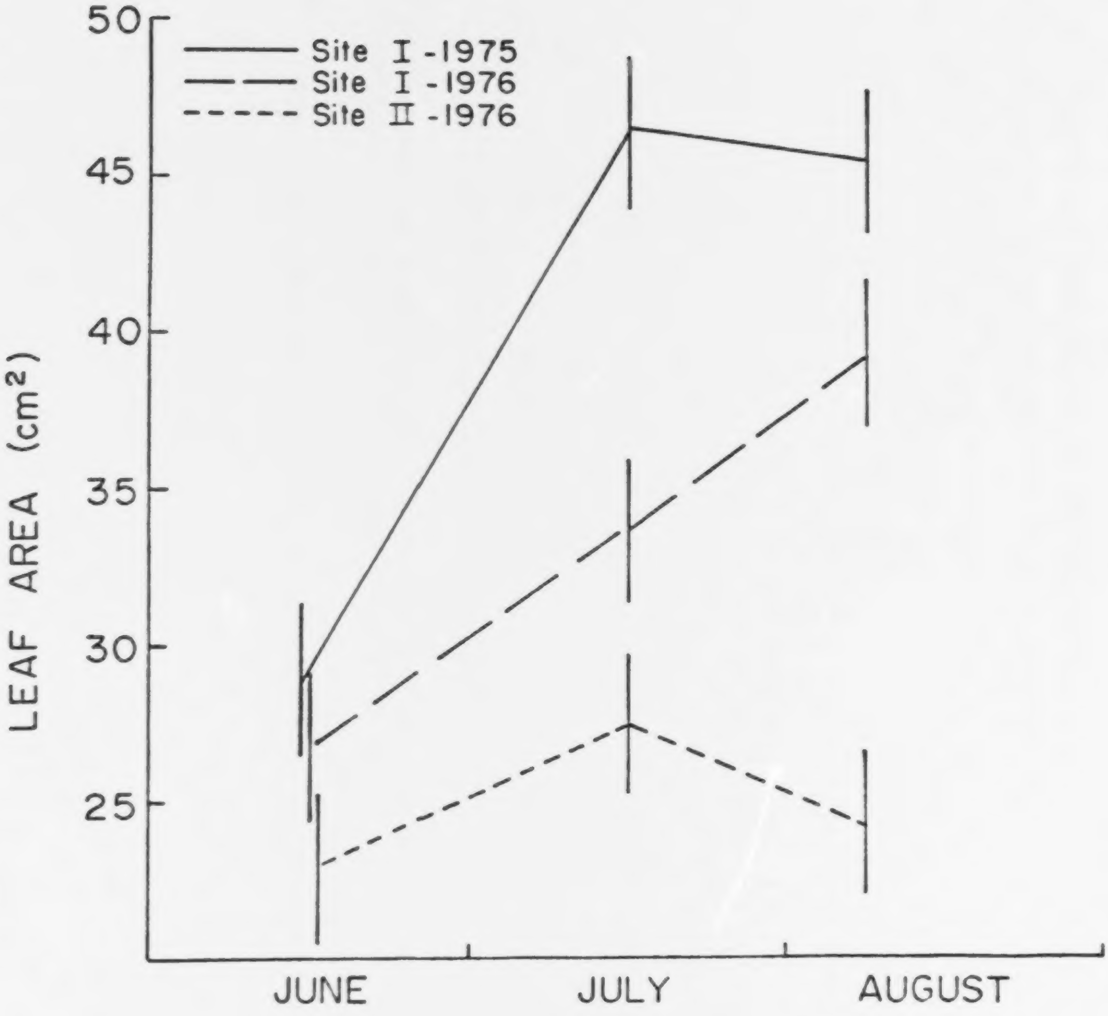


Figure 13.14. Two-way interaction effects of date and year-site on total leaf area (cm^2) for individual western wheatgrass plants. Means with overlapping vertical lines are not significantly different at $P = 0.05$ ($Q = 4.7$).

reported a marked decrease in the net photosynthetic rate of western wheatgrass when temperatures exceeded 20°C. In 1976 (Figure 13.12) average temperatures during this time interval were several degrees cooler than in 1975, and with ample soil water, plants on Site I continued to grow. However, on Site II plants did not continue growth into August because of greater water stress. The combined effect of these differences in growth conditions between 1975 and 1976 and between the two sites in 1976 was responsible for the significant year-site effect (Table 13.13).

The significant date × treatment interaction suggests SO_2 fumigation altered plant growth rates from June to August (Figure 13.15), although the only significant difference was in August when total leaf area of plants in the high treatment was significantly greater than in the control. However, a treatment effect was indicated by evaluating the rate of increase in total leaf area between each sample date. To evaluate these differences, we calculated mean relative leaf growth rates ($\overline{RLGR}$) ($cm^2 \cdot cm^{-2} \cdot week^{-1}$) for plants within each treatment between each sample date. The significant ($P < 0.01$) year-site and date effects (Tables 13.12 and 13.13) reflect differences in growing conditions between the 1975 and 1976 growing seasons and the two sites in 1976. In contrast to the total leaf area analyses, a significant ($P < 0.05$) treatment effect was indicated in $\overline{RLGR}$ (Table 13.14).

The effect of SO_2 treatment on $\overline{RLGR}$ can be more clearly understood by examination of the two-way interaction effect of date and treatment (Table 13.15). From the beginning of the growing season to mid-June, $\overline{RLGR}$ was similar in all treatments. From mid-June to mid-July $\overline{RLGR}$ of plants in the medium and high treatments was significantly greater than in the control and low treatments. From mid-July to early August $\overline{RLGR}$ was not significantly different between the control, medium, and high treatments. However, $\overline{RLGR}$ for plants in the low treatment was significantly greater than for plants in the control and medium treatments. In addition, lack of a significant year-site × treatment interaction suggested this trend occurred both years on Site I and on Site II in 1976. These results indicate a stimulation in leaf growth as a result of SO_2 exposure.

To determine if the increase in leaf area was the result of larger leaves, we compared the size of the four oldest leaves and their respective $\overline{RLGR}$. The significant ($P < 0.01$) date × leaf age × treatment × year-site interaction indicated no treatment differences. Therefore, we assumed the increase in total leaf area resulted from a greater number of leaves per plant.

Significant treatment, year-site, and date effects ($P < 0.05$) were revealed by the ANOVA for number of leaves, as well as significant date × treatment and date × year interactions. The date × treatment interaction (Figure 13.15) indicated plants in the low treatment averaged significantly fewer leaves in July than plants in the control, medium, and high treatments. However, by August plants in the control plots possessed significantly fewer leaves than those in treatment plots. The date × year-site interaction revealed a response similar to the total leaf area analyses except total number of leaves increased significantly from July to August in 1975 while total leaf area did not increase significantly. This apparent inconsistency

TABLE 13.13. MEAN VALUE OF WESTERN WHEATGRASS PLANTS FOR VARIOUS MEASURED PARAMETERS[*]

Parameters	Year-site		
	Site I 1975	Site I 1976	Site II 1976
Total leaf area (cm^2)	40.2_a[†]	33.3_b	24.8_c
Mean relative leaf growth rate ($cm^2 \cdot cm^{-2} \cdot week^{-1}$)	0.16_a	0.12_b	0.08_c
Number of leaves	5.0_a	4.6_b	4.0_c
Total necrotic leaf area (%)	31.6_a	4.1_a	34.8_a
Necrotic leaf area of four oldest leaves (%)	41.4_a	38.8_a	35.9_b
Biomass (mg)	180.0_a	230.0_b	--
Mean relative growth rate ($mg \cdot mg^{-1} \cdot week^{-1}$)	0.26_a	0.15_b	--

[*] Mean values are averages of control, low, medium, and high SO_2 treatments for June, July, and August sample dates. Biomass and mean relative growth rates based only on data from Site I in 1975 and 1976 and includes May, June, July, August, and September sample dates.

[†] Means in a row followed by same letter are not significantly different at $P = 0.05$.

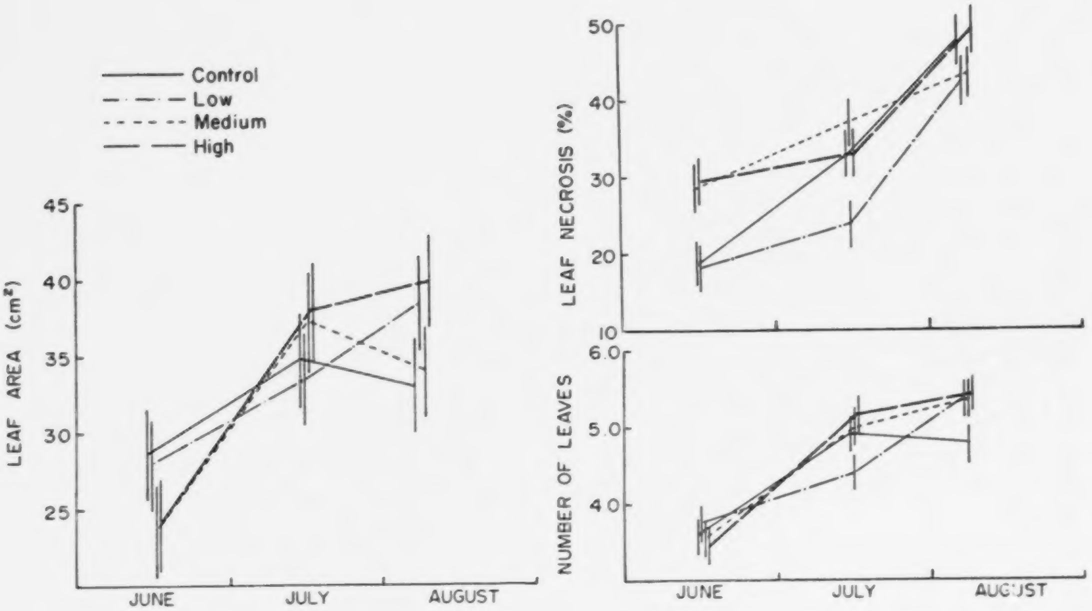


Figure 13.15. Two-way interaction effects of date and treatment on total leaf area (cm^2), total necrotic leaf area (%), and total number of leaves for individual western wheatgrass plants. Means with overlapping vertical lines on the same date are not significantly different at $P = 0.05$ ($Q = 6.0$) for total leaf area, at $P = 0.05$ ($Q = 6.1$) for total necrotic leaf area, and at $P = 0.05$ ($Q = 0.5$) for total number of leaves.

was attributed to shrinkage in leaf area from senescing tissue. The significant two-way interaction effects of date × treatment and date × year-site provided insight into the cause for the significant date, year-site, and treatment effects (Tables 13.12 to 13.14). Based on the close relationship between total leaf area and number of leaves per plant, it was apparent the suggested growth stimulation resulted from an increase in number of leaves per plant.

Visible Effects on Leaf Surfaces

Statistical analyses of percentage of necrotic leaf area per plant indicated significant ($P < 0.01$) treatment and date effects and a significant date × treatment interaction. Although significant differences did occur between plants in the low treatment and the medium and high treatments, none were significantly different from plants in the control and thus no leaf injury could be attributed to SO_2 fumigation (Table 13.14).

TABLE 13.14. MEAN VALUES OF WESTERN WHEATGRASS PLANTS FOR VARIOUS MEASURED PARAMETERS*

Parameters	Treatments (SO_2)			
	Control	Low	Medium	High
Total leaf area per plant (cm^2)	7.4_a†	7.6_a	6.9_a	7.3_a
Mean relative leaf growth rate ($cm^2 \cdot cm^{-2} \cdot week^{-1}$)	0.10_a	0.12_{ab}	0.12_{ab}	0.14_b
Number of leaves per plant	4.4_a	4.5_{ab}	4.6_{ab}	4.7_b
Total necrotic leaf area (%)	32.0_{ab}	27.8_a	36.2_b	37.0_b
Necrotic leaf area of four oldest leaves (%)	36.5_a	31.5_a	41.9_{bc}	44.5_c
Biomass (mg)	223.0_a	186.0_b	185.0_b	227.0_a
Mean relative growth rate ($mg \cdot mg^{-1} \cdot week^{-1}$)				

---

*Mean values are averages of Site I-1975, Site I-1976, and Site II-1976 for June, July, and August sample dates. Biomass and mean relative growth based only on data from Site I in 1975 and 1976 and includes May, June, July, August, and September sample dates.

†Means in a row followed by same letter are not significantly different at P = 0.05.

TABLE 13.15. TWO-WAY INTERACTION EFFECTS OF DATE AND TREATMENT ON MEAN RELATIVE LEAF GROWTH RATE FOR WESTERN WHEATGRASS PLANTS

	Mean relative leaf growth rate*		
Treatment	Beginning of growth to mid-June	Mid-June to mid-July	Mid-July to early August
Control	0.30_a*	0.04_a	-0.03_a
Low	0.30_a	0.03_a	0.03_b
Medium	0.29_a	0.11_b	-0.04_a
High	0.29_a	0.11_b	0.01_{ab}

*$cm^2 \cdot cm^{-2} \cdot week^{-1}$

The date × treatment interaction indicated a lower percentage of leaf necrosis for plants in the control and low treatments in June than for plants in the medium and high treatments (Figure 13.15). However, by July only plants in the low treatment had a significantly less amount of leaf necrosis and by August no differences were noted. The significant date effects reflected an increase in necrosis in all treatments with increasing plant age (Table 13.12).

Visual examination of leaf surfaces of treatment plants did not reveal any abnormal patterns of necrosis which could be attributed to SO_2 fumigation. Based on studies by Thomas (1956), Bleasdale (1973), and Bell and Clough (1973), we assumed an increase in leaf senescence would be the most probable mode of western wheatgrass to express visible injury from chronic SO_2 fumigation. In order to evaluate this hypothesis, an analysis of the percentage of necrotic leaf area of the four oldest leaves of each plant was undertaken.

Significant ($P < 0.01$) treatment, leaf age, and date effects were revealed in the ANOVA based on the four oldest leaves. Because of the complexity of the ANOVA model, numerous interactions were significant. Those interactions related to both date and leaf age were found to agree closely with the main effects of date and leaf age which suggested leaf necrosis increased with increasing leaf age and time (Table 13.12).

Treatment effects were similar to those found in the analyses for percentage of necrotic leaf area for the entire plant (Table 13.14). However, the addition of a significant difference between average percentage of necrotic leaf area for plants in the control and high treatments, based on the four oldest leaves of each plant, confirmed an increase in leaf necrosis as a result of SO_2 fumigation.

Examination of the significant three-way interactions more clearly elucidated treatment effects. The date × leaf age × treatment interaction generally indicated an increase in leaf necrosis with increasing SO_2 concentration for all ages of leaves in all months except July (Figure 13.16). In July, percentage of necrotic leaf area in the first (oldest leaf), second, and third leaves of plants in the low treatment was significantly less than for plants in the control or two higher treatments. The date × treatment × year-site interaction supported this trend for both 1975 and 1976 on Site I and 1976 on Site II.

Subtle Effects on Plant Growth

Because of differences in range condition among the four treatment plots of Site II, growth analyses were limited to Site I.

Analysis of variance for average dry weight biomass of individual plants for the May through September sample dates indicated significant ($P < 0.01$) year, treatment, and date effects and a significant year × date interaction. The year × date interaction emphasized differences in growing seasons between the two years. Because of earlier growth initiation in 1976, equivalent biomass was attained approximately one month earlier than in 1975. In addition, standing crop biomass began to decrease by September 1976. This was probably because of increased leaf shedding.

The significant year effects indicated greater biomass in 1976 than in 1975 when averaged across all dates (Table 13.13). The year × date interaction, however, suggested this conclusion was misleading with regard to peak biomass which was equal in both years. The significant treatment effect indicated individual plant biomass in the control and high plots was significantly greater than in the low and medium plots (Table 13.14). No explanation for these differences can be provided other than sampling variation in either biomass or density. The date effect indicated a continuation of growth until August with no significant growth between August and September (Table 13.12). Again, this emphasizes the characteristic growing season typical of this region.

The ANOVA for mean relative growth rates ($\overline{RGR}$) ($mg \cdot mg^{-1} \cdot week^{-1}$) indicated significant ($P < 0.01$) year and date effects and a significant year × date interaction. The year × date interaction revealed the cause for the significant year effect (Table 13.13). The higher $\overline{RGR}$ throughout the 1975 growing season resulted primarily from a higher $\overline{RGR}$ between growth initiation to mid-May in 1975 than in 1976. Although average biomass of individual plants was only 54 mg in May 1975, as compared to 102 mg in May 1976, the $\overline{RGR}$ was greater in 1975 than in 1976 since time from initial growth to mid-May was less in 1975 (31 days) than in 1976 (59 days). Mean relative growth rates decreased rapidly after mid-June in both 1975 and 1976 which emphasizes growing season characteristics.

Although no significant treatment effects were noted in the $\overline{RGR}$ analyses, we assumed possible differences in leaf area ratios (LAR) and aboveground net assimilation rates (NAR) may have resulted from the apparent stimulation in

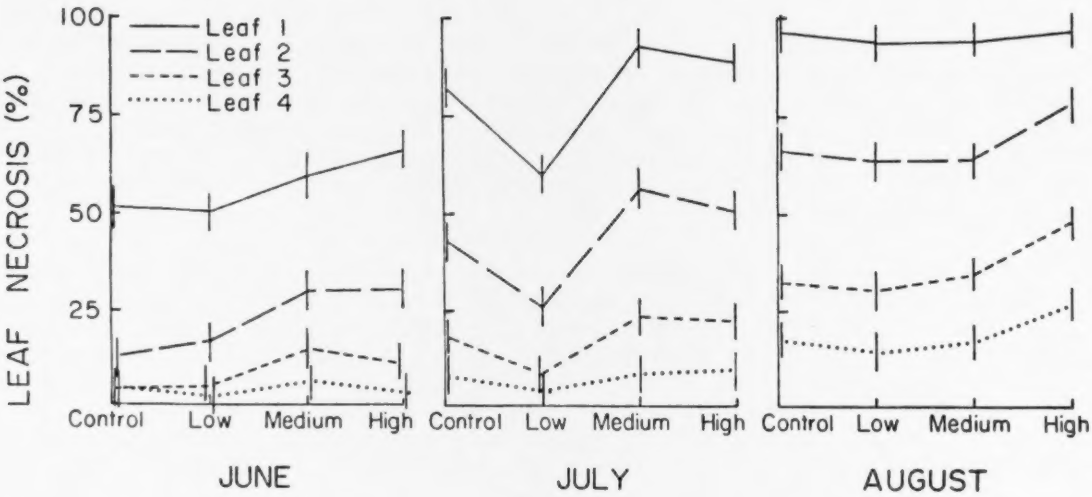


Figure 13.16. Three-way interaction effects of date, leaf age, and SO_2 treatment on percentage of necrotic leaf area for individual western wheatgrass plants. Oldest leaf is Leaf 1. Treatment means with overlapping vertical lines on same date are not significantly different at $P = 0.05$ ($Q = 10.4$).

leaf growth attributed to SO_2 treatment as indicated in the leaf area analyses. Statistical analyses of the average LAR and NAR for individual plants indicated no significant treatment effects although differences in dates and years were noted. These differences were attributed to temporal variations in growing seasons.

Discussion

Because of the relationships between subtle and visible effects of chronic SO_2 fumigations on western wheatgrass, discussion of the results pertinent to the first two objectives overlap. Thus, we discuss visible effects first and then relate these to subtle effects.

Recently, Tingey *et al.* (1976) exposed western wheatgrass plants, collected near our study areas, to acute levels of SO_2. Exposures at concentrations of 100 to 125 pphm SO_2 for 4 h were necessary to induce acute visible injury on leaf blade surfaces. This injury frequently occurred as small bifacial lesions and interveinal streaks of necrotic tissue ranging in color from light tan to ivory. Injury frequently developed first near the leaf tip of younger leaves and near the bend of the leaf in older leaves.

Visual examination of leaf surfaces in our study revealed no abnormal lesions or interveinal streaks which could be attributed to SO_2 treatment. An increase in leaf senescence was the only apparent visible injury incurred as a result of SO_2. Furthermore, the magnitude of this increase was insufficient to permit visual detection on individual plants. An increase in leaf senescence was first suggested in the analyses based on the entire leaf surface of each plant and was confirmed from the significant interaction of date × leaf age × treatment in the analysis of the four oldest leaves. Furthermore, it seems apparent this response was dependent on dosage of SO_2 received since it was related to both duration of exposure (date) and concentration (treatment).

Previous research investigating effects of chronic SO_2 exposures on perennial ryegrass has suggested an increase in leaf senescence resulting from SO_2 treatment (Thomas, 1956; Bleasdale, 1973; Bell and Clough, 1973). However, the mechanism whereby SO_2 may cause an increase in leaf senescence is not totally understood. It is generally accepted that after entry into the leaf, SO_2 is rapidly dissolved on the moist surfaces of the mesophyll cells to form sulfite which in turn is slowly oxidized to sulfate (Thomas *et al.*, 1950; Ziegler, 1975). Thomas and Hendricks (1956) reported sulfate was approximately 1/30 as toxic to a plant as was sulfite. It follows that as long as SO_2 is not absorbed at a rate exceeding the cell's capacity to oxidize sulfite to sulfate, sulfite is prevented from reaching toxic levels. However, as the concentration of sulfate increases over time, toxic levels of sulfate are eventually attained (Malhorta and Hocking, 1976) and thus, increased leaf senescence is observed.

Our data suggest that until approximately mid-June, western wheatgrass plants grew at a rate sufficient to incorporate the SO_2-sulfur into normal metabolites, thereby preventing toxic levels of sulfite or sulfate from accumulating. Lack of a treatment effect in May 1976 emphasizes the importance of rapid growth rates. Sulfur dioxide treatments were initiated approximately six weeks later in 1975 than in 1976 and therefore, significantly greater dosages of SO_2 were encountered on corresponding dates in 1976 than in 1975. Treatment means for the percentages of necrotic leaf area on Site I were not significantly different between June 1975 and June 1976. Thus, it is apparent sulfate was prevented from reaching toxic levels by incorporation into normal metabolic processes and leaf senescence was therefore delayed. Recently, Schwartz *et al.* (1978) (see preceding section) reported increasing concentrations in total sulfur with increasing SO_2 concentrations in western wheatgrass tissue subsampled from our harvest samples of live tissue. Their data indicate a sharp increase in total sulfur concentrations after mid-June with similar concentrations in June of both 1975 and 1976.

Previous research has indicated plants are most sensitive to SO_2 injury when conditions are most favorable for optimal growth (Katz, 1949; Daines, 1968). However, data supporting this generalization were primarily attained from acute levels of exposure rather than from chronic levels similar to those utilized in this study. The importance of optimal growth rates in acute exposures is to provide an avenue of ingress of SO_2 into the plant through the open stomata. Our results suggest that rapid growth prevented sulfate from attaining a toxic concentration. Once metabolic activity began to slow, either because of maturation of older leaves or adverse growing conditions, toxic levels of sulfate were assumed to have accumulated. Both growing seasons were characterized by a wet season followed by a dry season with a rather abrupt transition occurring during July. Therefore, the time period of moderate growth rates was limited and low levels of SO_2 fumigation were insufficient for toxic levels of sulfate to accumulate. Thus, plants in the low SO_2 treatment did not express an acceleration in leaf senescence although plants in the medium and high treatments did experience increased senescence.

The decrease in percentage of senescing leaf tissue of the three oldest leaves in the low treatment in July (Figure 13.16) on Site I in 1975 and 1976 and Site II in 1976 is perplexing. Two explanations for this decrease seem plausible: (1) inherent differences in growing conditions between the control and treatment plots accelerated leaf senescence in the control plants, or (2) a stimulation in leaf growth, as a result of SO_2 treatment, reduced leaf senescence in the low treatment.

The first explanation is formulated strictly on field observations during the two years of data collection. On Site I we assume differences in available soil water existed between treatment plots. On Site II differences in available soil water between the control and low treatment and possible differences in previous grazing intensities are assumed to have existed. The following observations support these assumptions.

Several large areas of slight surface depressions were noted in the control plot on Site I. These areas were assumed to have resulted from previous removal of top soil either by wind or water. Surface soils within these areas had a higher clay content than soils throughout the remainder of the plot. In addition, vegetation within these areas generally reflected greater water stress than adjacent vegetation. It is assumed the high clay content of the soil in these areas induced greater water stress on the vegetation and a reduction in growth and increased leaf senescence resulted. This was most evident in July since growing conditions in June were closer to optimal than in July and considerable water stress masked differences between all treatments in August. Although these areas were avoided when sampling, their influence may have exceeded visual detection.

Similarly, on Site II differences in total soil water discussed previously may have caused decreased growth and accelerated leaf senescence. In addition, visual examination of the control plot suggested heavy grazing occurred on the plot prior to fencing in 1975. Therefore, a possible decrease in plant vigor in 1976 could be expected (Tomanek and Albertson, 1953; Laycock, 1967). Again, increased leaf senescence and a reduction in growth may have resulted.

Based on the above observations, interpretation of the treatment effects of this study is significantly altered from an interpretation disregarding any inherent difference between treatment plots. The significant date × treatment interaction in the analyses of the total leaf surface, number of leaves, and percentage of necrotic leaf area for individual plants supports a reduction in growth on the controls and increased leaf senescence near mid-July (Figure 13.15). In addition, the reduced senescence displayed in the low treatment in July can be hypothesized to have resulted from an abnormal but relatively higher rate of senescence in the control plants. The critical dosage required to induce an acceleration in leaf senescence probably was not attained in the low SO_2 treatment until after the July sample date. However, it is questionable whether SO_2 dosage in the low treatment was ever sufficient to induce increased leaf senescence since no significant differences were apparent between plants in the control and low treatments. In addition, leaf senescence was higher in the control plot than the treatment plots in August 1976 on Site I. This fact certainly adds credence to this explanation.

The second explanation, involving a stimulation in leaf growth because of SO_2 treatment, was indicated by analyses of average number of leaves for individual plants and average $\overline{\text{RLGR}}$ during June, July, and August. Furthermore, the treatment × date interactions, discussed previously, provided support for this explanation.

Total leaf surface of the control plants increased at a relatively constant rate from June to August (Figure 13.15). However, a period of accelerated leaf growth was evident from June to July in the medium and high treatments and from July to August in the low treatment. These periods of accelerated growth were primarily the result of an increase in number of leaves and not larger leaves. Lack of significant treatment × year interactions suggests these periods of accelerated growth were replicated in all three year-sites. Furthermore, the presence of significant treatment effects in the ANOVA for number of leaves and $\overline{\text{RLGR}}$ supports a stimulation of leaf growth because of SO_2 treatment. Treatment means indicate increasing number of leaves and increasing $\overline{\text{RLGR}}$ with increasing SO_2 concentration (Table 13.14).

Although inherent differences in growing conditions may have occurred between the control and treatment plots, evidence of a stimulation in leaf growth with increasing SO_2 persists when data from control plots are disregarded. Thus, we conclude leaf growth was stimulated by chronic SO_2 fumigations.

At least two explanations can be hypothesized whereby a stimulation in growth, as a result of SO_2 fumigation, appears logical. The first explanation requires extension of the dosage response, which we concluded increased leaf senescence, to include a dosage response stimulating leaf growth. Since rapid leaf growth occurred near the time of accelerated senescence, we suggest SO_2 directly stimulated leaf growth at a dosage near that required to cause an increase in senescence. Since the threshold dosage required to induce accelerated leaf senescence would be influenced by tissue age and exposure time, analyses of the percentage of necrotic leaf area for the entire plant render support to this hypothesis. Because of accelerated growth from June to July in the medium and high treatments, leaf senescence by mid-July was similar in the control, medium, and high treatments (Figure 13.15). This resulted because a proportionally greater quantity of young leaf tissue was present in the treatment plots. Percentage of total necrotic leaf area was thereby reduced since dosage was insufficient to accelerate senescence in the younger leaf tissue. However, dosage was sufficient to accelerate senescence in the older leaves (Figure 13.16).

The reduced senescence displayed by the four oldest leaves of the low treatment plants in July is hypothesized to have resulted from the interaction effects of simultaneous accelerated growth and senescence. Since leaf senescence begins at the leaf tip and gradually advances toward the base, it is apparent that age of leaf tissue is important in normal senescence. It follows that tissue at the leaf tips receives a greater dosage of SO_2 than the younger tissue near the intercalary meristem at the leaf base. We have measured higher concentrations in total sulfur in leaf tips than in bases in fumigated plants (Ziegler, 1975). We assume tissue near the leaf tip received a dosage of SO_2 by July, sufficient to induce accelerated senescence while tissue near the base received a dosage sufficient to stimulate growth.

The interaction of decreasing leaf area because of senescence and increasing leaf area because of growth may possibly account for the lack of any significant treatment effects on size of leaves. Average $\overline{\text{RLGR}}$ from June to July for the four oldest leaves was -0.027, 0.002, 0.016, and 0.010 $cm^2 \cdot cm^{-2} \cdot week^{-1}$ in the control, low, medium, and high treatments, respectively. Although these rates were not significantly different, they do provide trend information whereby the July data can be explained. We assume leaves of the control plants were not growing at a sufficient rate to counteract the shrinkage accompanying normal leaf senescence and thus, an overall negative rate of leaf growth resulted. In the high treatment, leaf senescence occurred at an accelerated rate, because of dosage, but leaves were also growing at an accelerated rate sufficient to counteract shrinkage and thus an overall positive increase in leaf area occurred. This was also true in the medium treatment. In the low treatment, rate of growth was sufficient to nullify the effect of senescence and therefore percentage of necrotic leaf area was low.

For SO_2 to act as a growth stimulant, we assume SO_2-sulfur can act as a source for normal sulfur requirements. Previous research has indicated at least a portion of a plant's sulfur requirements can be met by direct uptake of SO_2 if present at very low concentrations (Faller, 1971; Bromfield, 1972; Cowling *et al.*, 1973; Cowling and Lockyer, 1976).

From these studies it is apparent SO_2 can act as a source of sulfur, particularly when sulfate in the growing medium is inadequate to meet normal sulfur requirements of a plant. Furthermore, under conditions of adequate sulfur nutrition, low levels of SO_2 appear to possibly stimulate aboveground growth (Faller, 1970, 1971). Nonsignificant increases have been reported in the grain yield of little club wheat (*Triticum aestivum* L.) (Swain and Johnson, 1936) and the dry matter yield of alfalfa (Thomas *et al.*, 1943) when plants were exposed to low levels of SO_2 in the presence of adequate sulfur nutrient.

Contrary evidence suggesting a depression of perennial ryegrass yield when exposed to low concentrations of SO_2 has been provided by Bell and Clough (1973). Plants exposed to 6.7 pphm SO_2 throughout a 26-week growth period reduced number of tillers by 41%, number of leaves by 44%, dry weight of live leaves by 50%, and leaf area per plant by 51% when compared to control plants. Similar results were reported during both summer and winter growing conditions. However, six weeks after sowing, when plants were mostly at the three-leaf stage, length of the second leaf of the control and SO_2 treatment plants was measured. These measurements indicated treatment plants had a slight but significantly larger second leaf than control plants. No explanation was provided for this apparent stimulation in leaf size.

Bleasdale (1973) reported plants grown in continuously scrubbed air exceeded the yield of plants grown in continuously polluted air by 20% to 135%. In addition, number of leaves and number of tillers per plant were significantly reduced when plants were grown in continuously polluted air. However, when plants were exposed to polluted air for only a portion of each day, marked increases in dry-weight, in number of leaves, and in number of tillers per plant were noted. The author suggested intermittent exposure to SO_2 enhanced cell division by interfering with the balance between oxidized and reduced sulfur radicals which Hammett (1930) suggested was an important factor in controlling normal cell division. Similar conditions may have promoted the increase in number of leaves per plant associated with increasing SO_2 in our study. Sulfur dioxide concentrations during nighttime often exceeded daytime concentrations by a factor of ten because of reduced wind velocities and reduced thermal uplift during the night.

Since soils within our study areas are not considered sulfur deficient, our results appear to conflict with the two studies above. However, both studies do suggest some possible stimulation in leaf growth which may be applicable here. It should also be noted that ryegrass is considered a sensitive species to SO_2 fumigation whereas the results reported by Tingey *et al.* (1976) indicate western wheatgrass is more resistant.

It may also be hypothesized that the stimulation in growth was the indirect result of increased senescence. It has been well documented that net photosynthetic rates change as leaves age (Thorne, 1963; Moss and Peaslee, 1965; Jewiss and Woledge, 1967; Lupton, 1968; Risser and Johnson, 1973). Since normal leaf senescence is dependent on leaf age, it may be postulated that removal of the oldest leaves would increase the average photosynthetic rate of the plant. If average photosynthetic rate was increased, then the resulting effect might be a stimulation in growth. Kulman (1971) noted an increase in aboveground growth of trees when the older, less photosynthetically active leaves were removed. Thus it can be hypothesized that the increased rate of senescence caused by SO_2 increased the average photosynthetic rate of western wheatgrass and this in turn stimulated shoot growth.

It has also been shown that senescing leaves act as a net exporter of certain minerals and metabolites (Brady, 1973). For example, it has been estimated 90% of the nitrogen and phosphorus and 70% of the potassium may eventually be transported from senescing leaves (Williams, 1955). Thus, various hypotheses may be postulated as to why accelerated leaf senescence might stimulate leaf production.

The third objective was to evaluate the effects of chronic SO_2 fumigations on relative growth rates, net assimilation rates, and leaf area ratios of individual plants. Although alterations in total leaf area and rate of senescence were attributed to SO_2 treatment, the magnitude of these alterations was insufficient for detection at the plant level of organization.

However, it must be emphasized that the sensitivity of our growth analyses was at a level where changes of rather large magnitudes would be necessary for detection of significant differences. Although alterations in growth parameters probably occurred at various times between each sample date, the accumulative effects were not detectable because of the variation in sample estimates. In addition, only aboveground growth was examined and it is feasible that major treatment differences in RGR and NAR may have occurred in the belowground portion of each plant.

Summary of 1975 and 1976 Studies

The effects of three different concentrations of sulfur dioxide (SO_2) on western wheatgrass (*Agropyron smithii* Rydb.) in a Montana grassland were studied. The response of western wheatgrass was examined at three levels of organization: organ (leaf), organism (plant), and community.

Forty western wheatgrass plants were collected from each treatment in June, July, August, and September 1975 and in May, June, July, and August 1976. Leaves were removed and mounted between two clear pieces of acetate and total adaxial leaf area and necrotic area were recorded. Aboveground biomass was sampled monthly, by the harvest method, over the 6-month growing season. Abiotic parameters monitored were: precipitation, soil water, temperature, relative humidity, and SO_2 concentrations.

It was concluded that SO_2 fumigation stimulated leaf growth. This stimulation was reflected by greater numbers of leaves per individual western wheatgrass plant rather than larger leaves. Increased leaf number appeared to be directly related to both time and concentration of SO_2 exposure (dosage response). Two hypotheses were advanced as plausible explanations for stimulation of leaf production. The first was based on the assumption that SO_2 directly stimulated growth while the second suggested stimulation was an indirect effect of SO_2.

No abnormal lesions on leaf surfaces were evident which could be attributed to SO_2 treatment. However, an increase in leaf necrosis was concluded to have occurred with increasing SO_2 treatment. The increased necrosis was not visibly distinguishable from normal leaf senescence.

Examination of the response of individual plants indicated no significant alterations in aboveground relative growth rates, net assimilation rates, or leaf area ratios. However, it was suggested that caution be exercised when relating these conclusions to long-term effects because of the magnitude of variability associated with the analyses of the responses at the organism and community levels or organization.

Growth and Senescence (1977)

Data collection procedures were altered for the 1977 growing season to accommodate a change in emphasis of our research program from biomass dynamics and primary production to physiological responses. Because we would no longer be sampling biomass at frequent intervals during the growing season, estimates of growth and senescence for 1977 are based on repeated measurements on 20 plants in each treatment plot. Measurements of total height, length, and width at the base of each leaf, length of green tissue on each leaf, and total number of leaves were made at c[illegible] two-week intervals from 13 April to 17 August. On 17 August each pla[illegible] harvested, labeled, and stored for chemical analysis. Leaf area was calculated by assuming that the leaves were triangles. This certainly results in a small error since the leaves are not exact triangles. Since there is no indication that leaf shape is altered by SO_2 either from the literature or our own work, we believe that this is a valid method to compare treatments.

Because we have not had time to carefully analyze these data, the results presented here will be limited to those that correspond to the significant findings from the 1975 to 1976 work. These are significant increases in the number of leaves per plant and in leaf senescence with increasing SO_2 concentration.

Leaf numbers for the control and treatments on Site I indicated no differences throughout the growing season (Figure 13.17). In addition, there were no differences in the timing of leaf development. Results for Site II were similar except that there was a slight increase in leaf numbers on the 5 and 10 pphm treatments (Figure 13.18).

Figures 13.19-13.26 illustrate the dynamics of leaf area for leaves 1-4 on Site I and II, respectively. The generalization that can be made from all of these data is that SO_2 causes senescence to begin earlier and reduces the functional life of the leaves of western wheatgrass. Leaf 2 from Site I may be used as an example to illustrate both of these points. The first dates when measurable dead material was observed were 28 May, 28 May, 9 May, and 25 April for the control, low, medium, and high treatments, respectively. The dates of total senescence in the same order were 28 July, 28 July, 23 June, and 10 June. These dates were selected using overlap of the standard error bars as an indication of no significant difference. The functional lives of these leaves were then 106, 106, 71, and 58 days. This is only a very rough expression of the functional lives of these leaves because the data also indicate increasing SO_2 increased the rate that 100% senescence was approached. Once we begin to understand the relationships of leaf age and SO_2 to photosynthesis of western wheatgrass, we will be in a better position to describe the functional significance of increased senescence.

EFFECTS OF CONTROLLED LEVELS OF SO_2 ON PHYSIOLOGY OF WESTERN WHEATGRASS

Investigations begun in 1977 emphasized physiological responses of western wheatgrass both under field and laboratory conditions. Variables measured in the field included air temperature, relative humidity, wind speed, solar radiation, leaf water potential, stomatal conductance, and $^{14}CO_2$ fixation. Laboratory investigations are just beginning at this time and variables to be monitored are air temperature, dew point temperature,

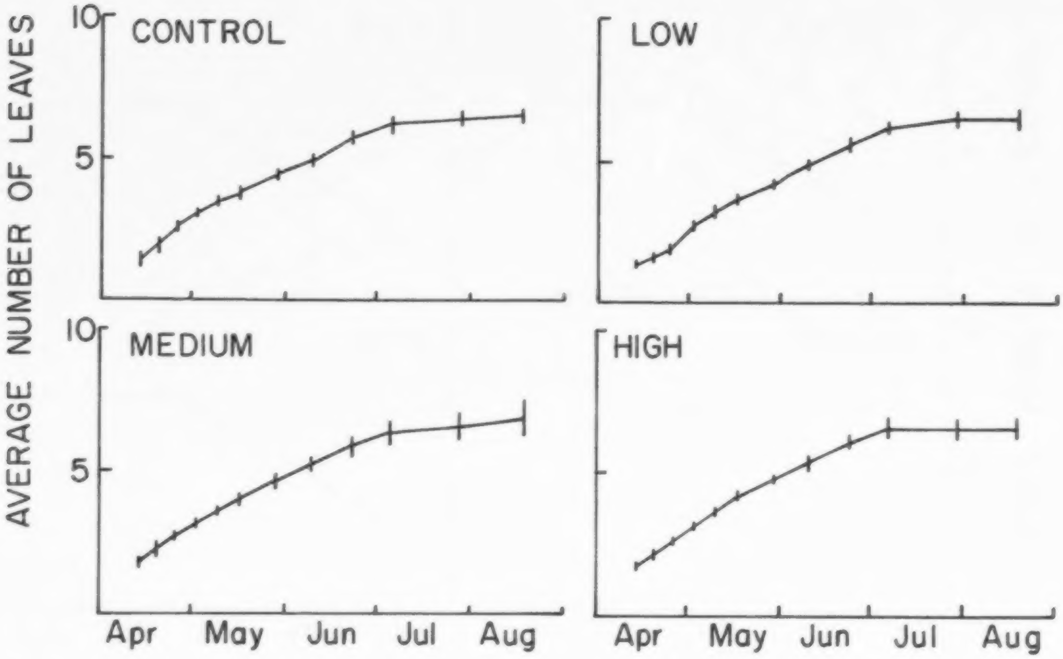


Figure 13.17. Mean number of leaves per plant, ± 1 SE, ZAPS I, 1977.

light intensity, sulfur dioxide concentration, stomatal conductance, transpiration, photorespiration, dark respiration, and net photosynthesis.

Methods

Field Investigations

Carbon fixation under control and high SO_2 concentrations (10 pphm) was measured using $^{14}CO_2$ and a labeling technique described by Shimshi (1969). Intact leaves are exposed to $^{14}CO_2$ for a short period of time (15 s), harvested immediately and placed in a test tube on dry ice to minimize loss of ^{14}C as a result of respiration. Samples are taken to the laboratory and prepared for liquid scintillation courting by the procedures described by Tieszen *et al.* (1974).

Stomatal conductance was measured with a mass flow porometer modified for use on narrow leaf grasses. Leaf water potential was measured with a pressure bomb (Scholander *et al.*, 1965). Water potential was measured on leaves immediately following exposure to $^{14}CO_2$.

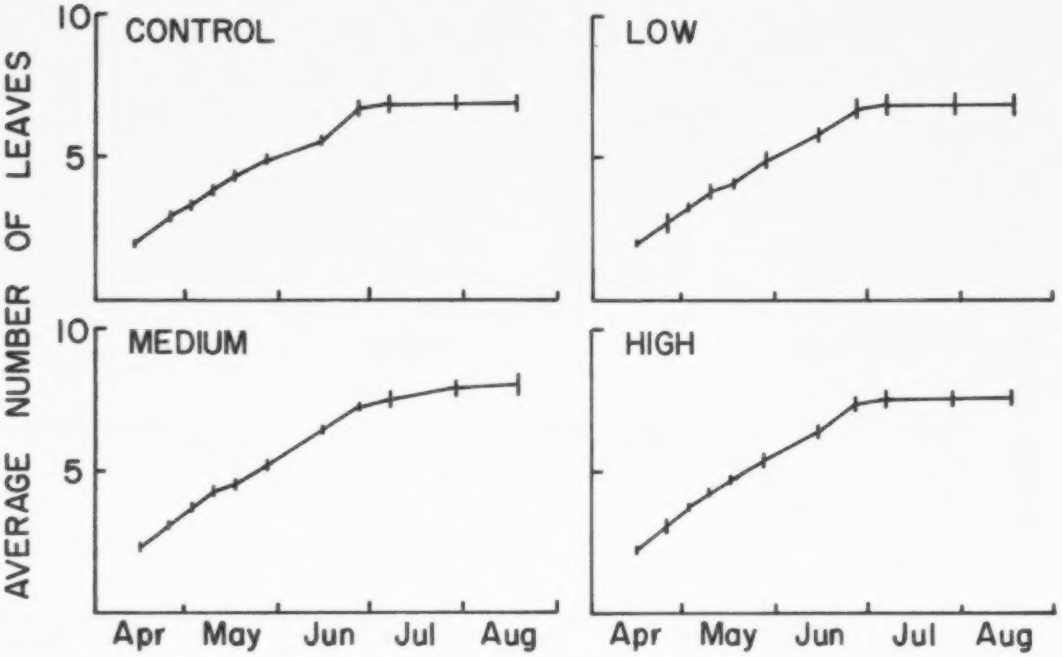


Figure 13.18. Mean number of leaves per plant, ± 1 SE, ZAPS II, 1977.

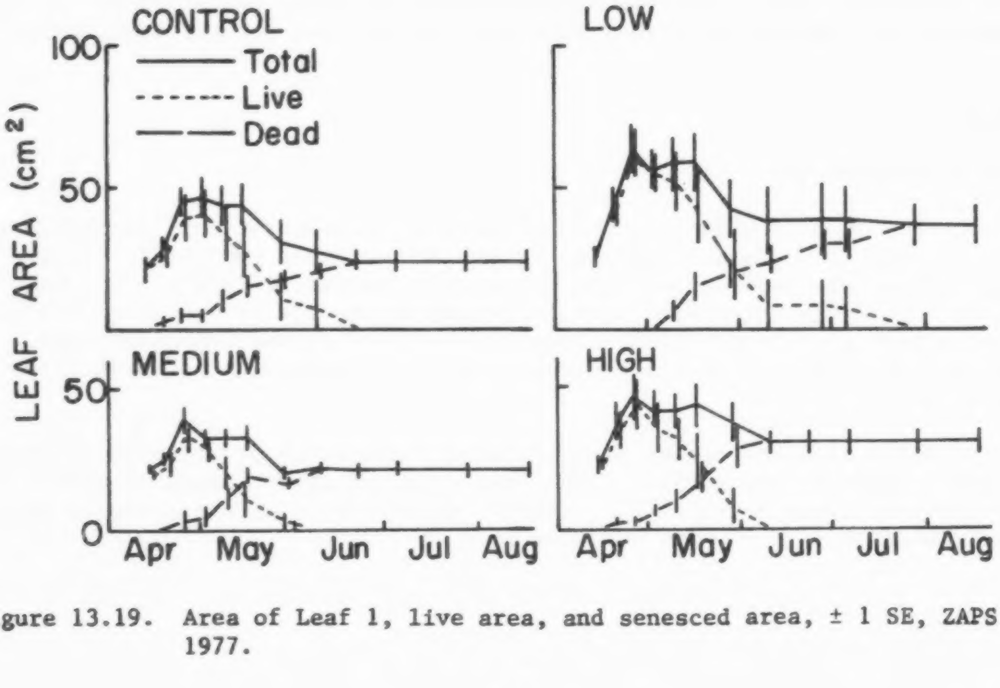


Figure 13.19. Area of Leaf 1, live area, and senesced area, ± 1 SE, ZAPS I, 1977.

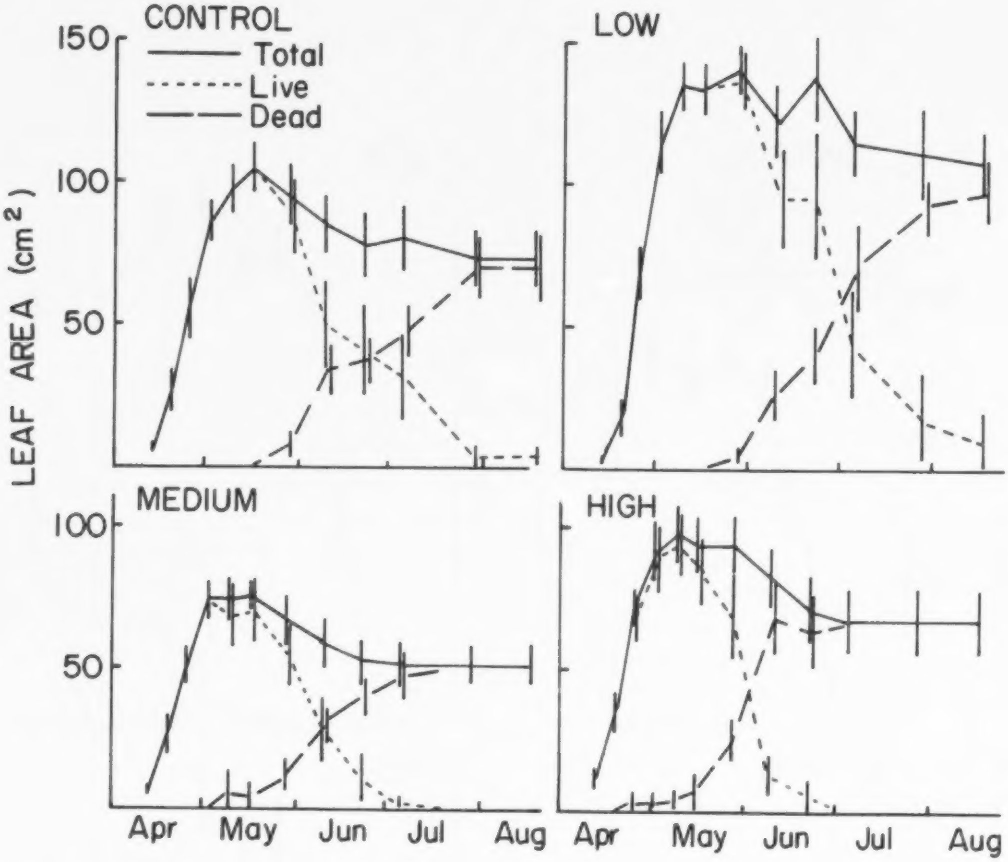


Figure 13.20. Area of Leaf 2, live area, and senesced area, ± 1 SE, ZAPS I, 1977.

Description of the Laboratory CO_2 Exchange System (Figure 13.27)

Air is supplied by the laboratory compressor system. The air is then pressure-regulated to a low value and scrubbed clean of all CO_2, moisture, ozone, and any other impurities using molecular sieves. An adjustable humidifier provides the capability to adjust the dew point of the air stream to any desired value. Carbon dioxide gas is then added to the air stream to provide a 310-ppm concentration using a "mixing tee" which was designed to provide complete mixing of the CO_2 into the carrier air stream. Sulfur dioxide is then added to the air stream through another (stainless steel) mixing tee. A temperature controller will provide flexibility in regulating the air stream temperature.

The gas stream then is split into two branches. The first branch goes to the Beckman Infrared Gas Analyzer (IRGA) reference cell for determination of the CO_2 content of the input stream. The second branch is routed through

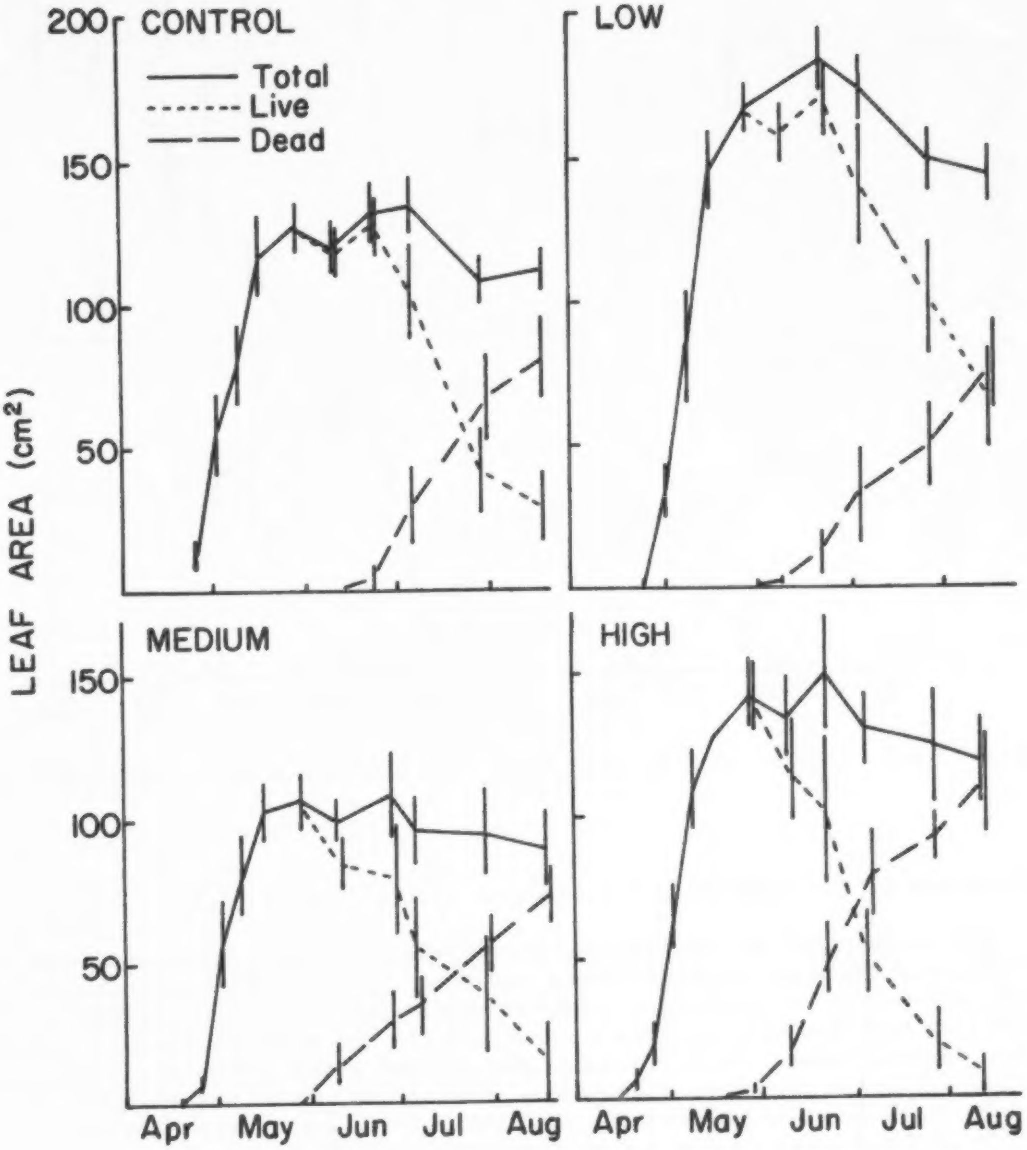


Figure 13.21. Area of Leaf 3, live area, and senesced area, ± 1 SE, ZAPS I, 1977.

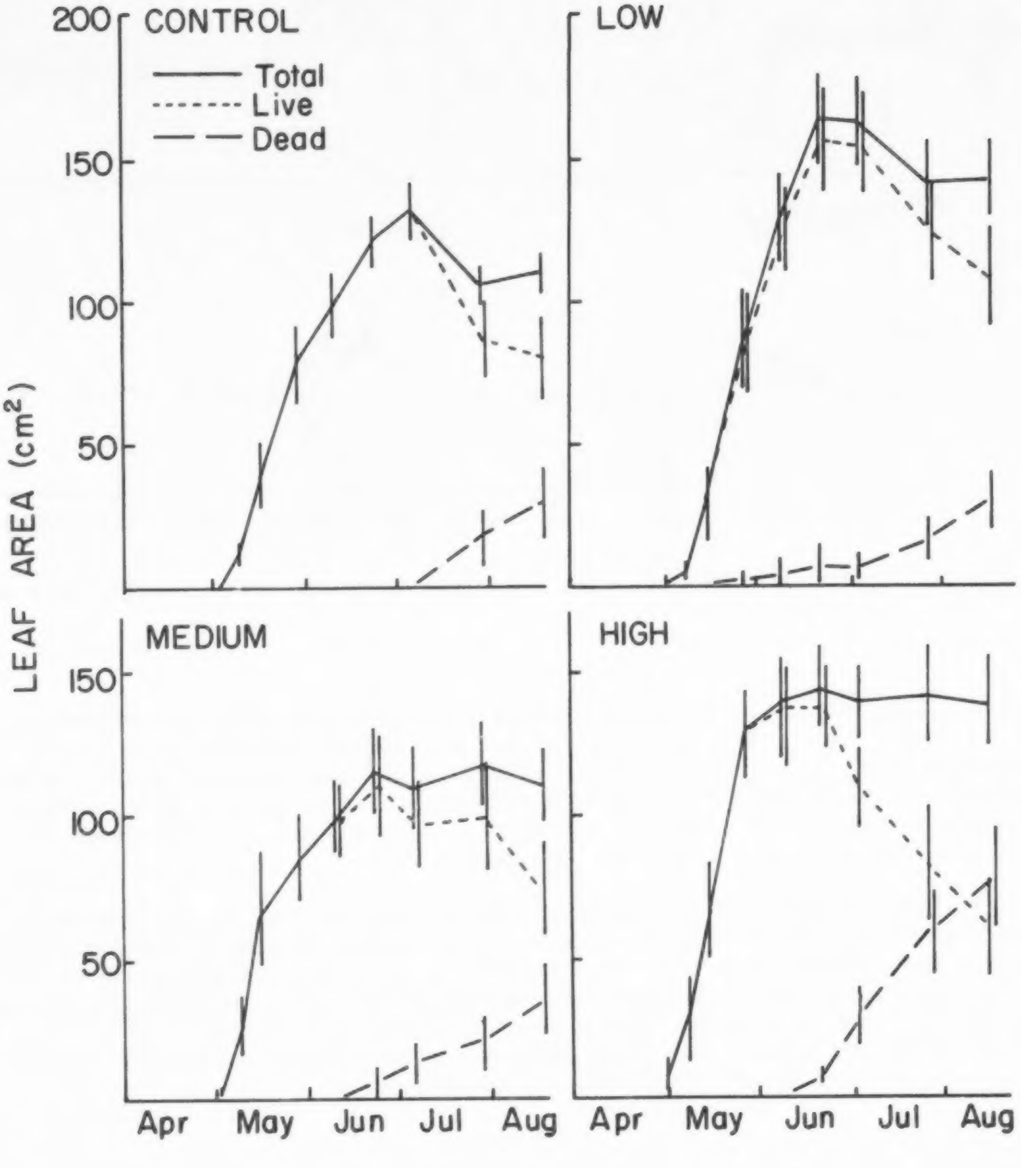


Figure 13.22. Area of Leaf 4, live area, and senesced area, ± 1 SE, ZAPS I, 1977.

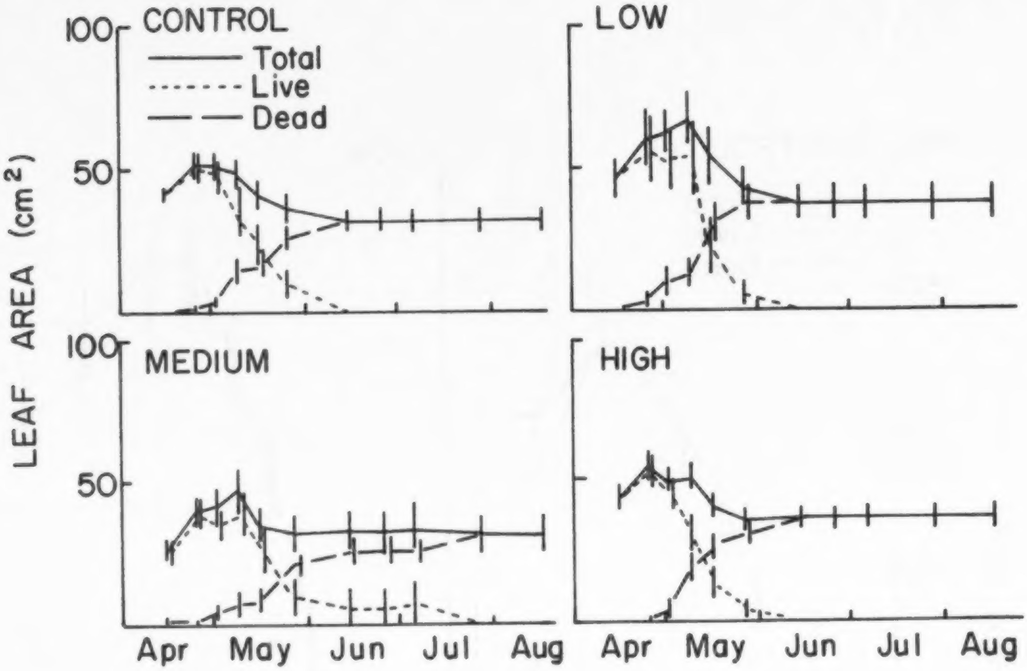


Figure 13.23. Area of Leaf 1, live area, and senesced area, ± 1 SE, ZAPS II, 1977.

a Plexiglass leaf cuvette situated under an adjustable 1000-watt Holophane sodium lamp. Temperature control for the cuvette is provided by a heat-absorbing water layer filter between the lamp and the cuvette, and by water circulated from a Masterline temperature-controlled bath to a water jacket in the cuvette. After passing through the cuvette, part of the stream is monitored for CO_2 content by the IRGA sample cell.

In order to monitor the humidity and SO_2 content of the air stream entering and exiting the cuvette, samples are taken from both sides of the cuvette and routed, by a time-sharing device, to a dew point hygrometer and an SO_2 analyzer. The IRGA requires a stream of air with constant CO_2 concentration to serve as a purge, and this is provided by a division of the air stream after the scrubbers. When each stream of air has served its purpose, it is routed to a "dump" where it is disposed.

Results

Field Investigations

Since field studies have been in progress for only one field season, only a sample of the information we are collecting will be presented here. Average diurnal patterns of environmental variables, leaf water potential,

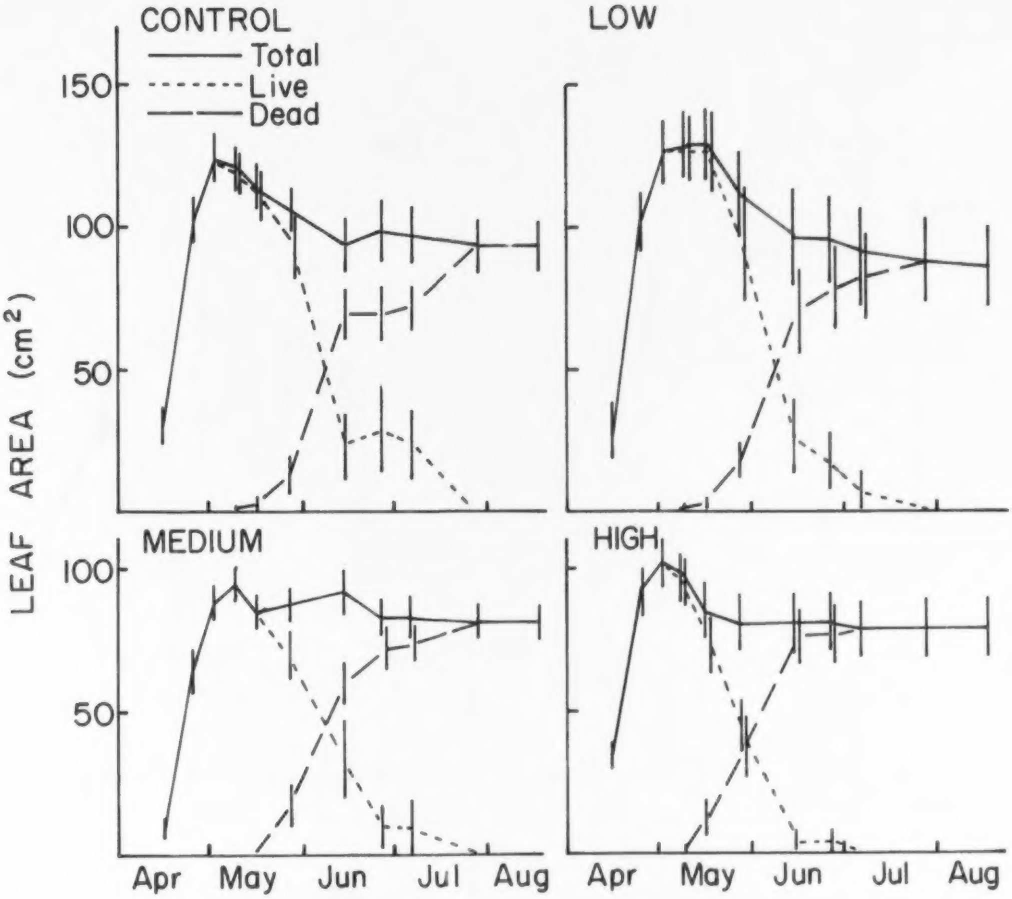


Figure 13.24. Area of Leaf 2, live area, and senesced area, ± 1 SE, ZAPS II, 1977.

and $^{14}CO_2$ uptake for a 3-day period in early July are presented for the control and high SO_2 treatment in Figure 13.28. Maximum air temperature was 23°C, occurring at 1600. Winds were light and variable ranging from zero at 0600 to $3\ m \cdot s^{-1}$. Predawn relative humidites averaged 75% and decreased to 30% at 1000 and remained constant throughout the remainder of the day. Maximum radiation was $70\ cal \cdot cm^{-2} \cdot h^{-1}$ at 1500.

Predawn leaf water potentials were greater than -5 bars for both the control and high treatment. Leaf water potentials decreased rapidly reaching -20 bars by 0700 for the control. Minimum leaf water potentials were -35 bars for the control at 1400 and -30 for the high treatment at 1600.

Uptake of $^{14}CO_2$ was maximum for the control between 0800 and 0900 at 13 mg $^{14}CO_2 \cdot dm^{-2} \cdot h^{-1}$ and remained low for the remainder of the days. The

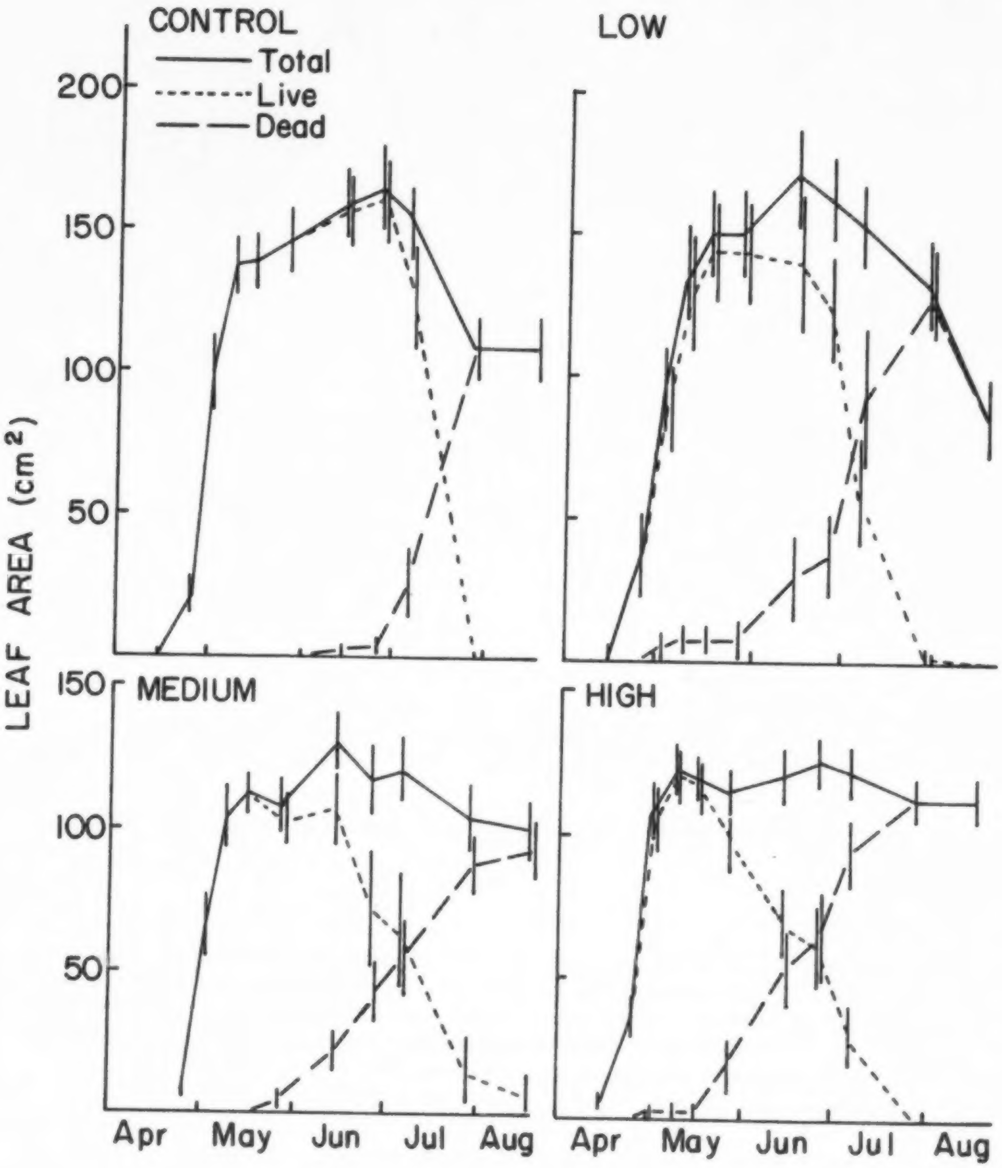


Figure 13.25. Area of Leaf 3, live area, and senesced area, ± 1 SE, ZAPS II, 1977.

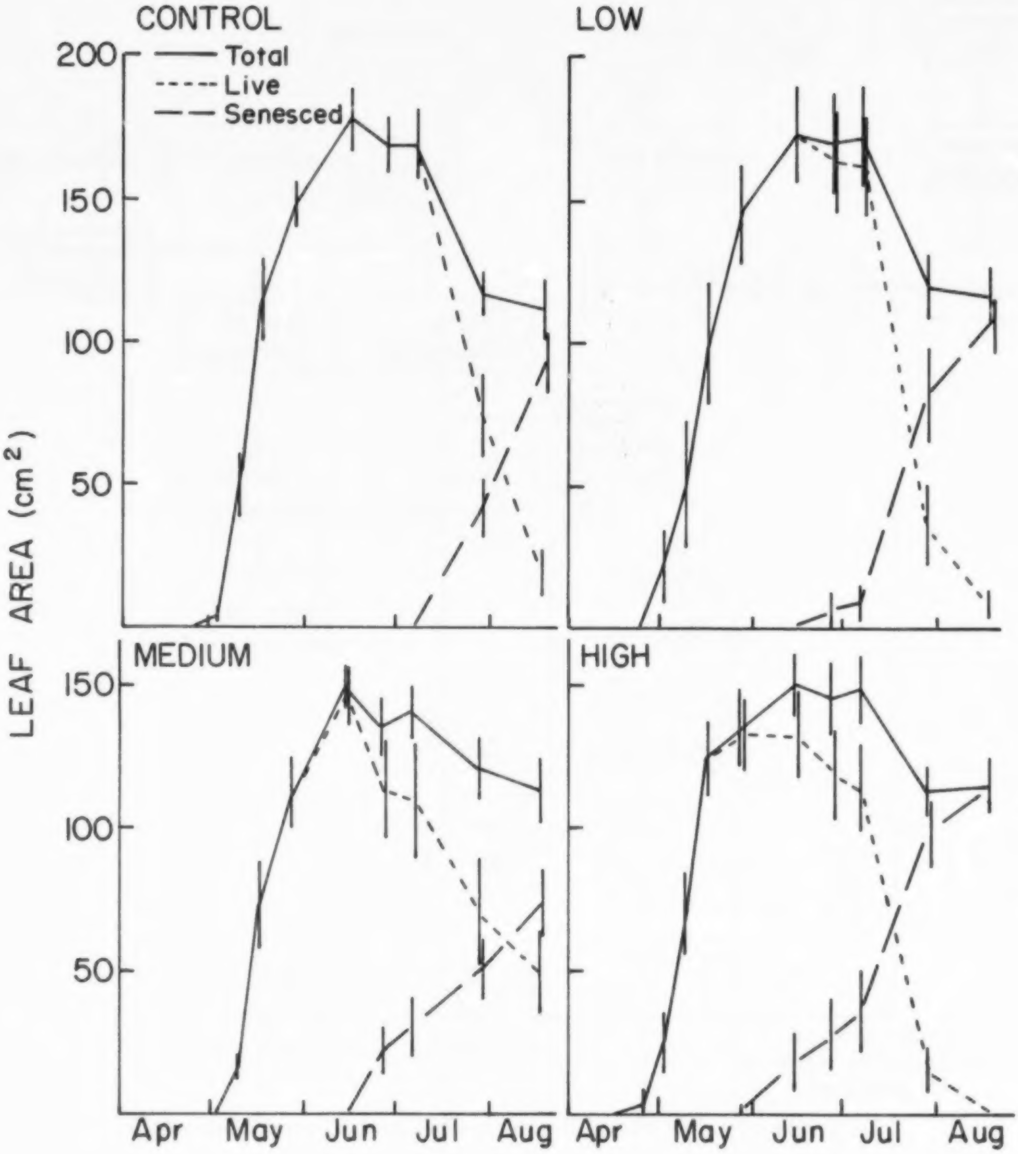


Figure 13.26. Area of Leaf 4, live area, and senesced area, ± 1 SE, ZAPS II, 1977.

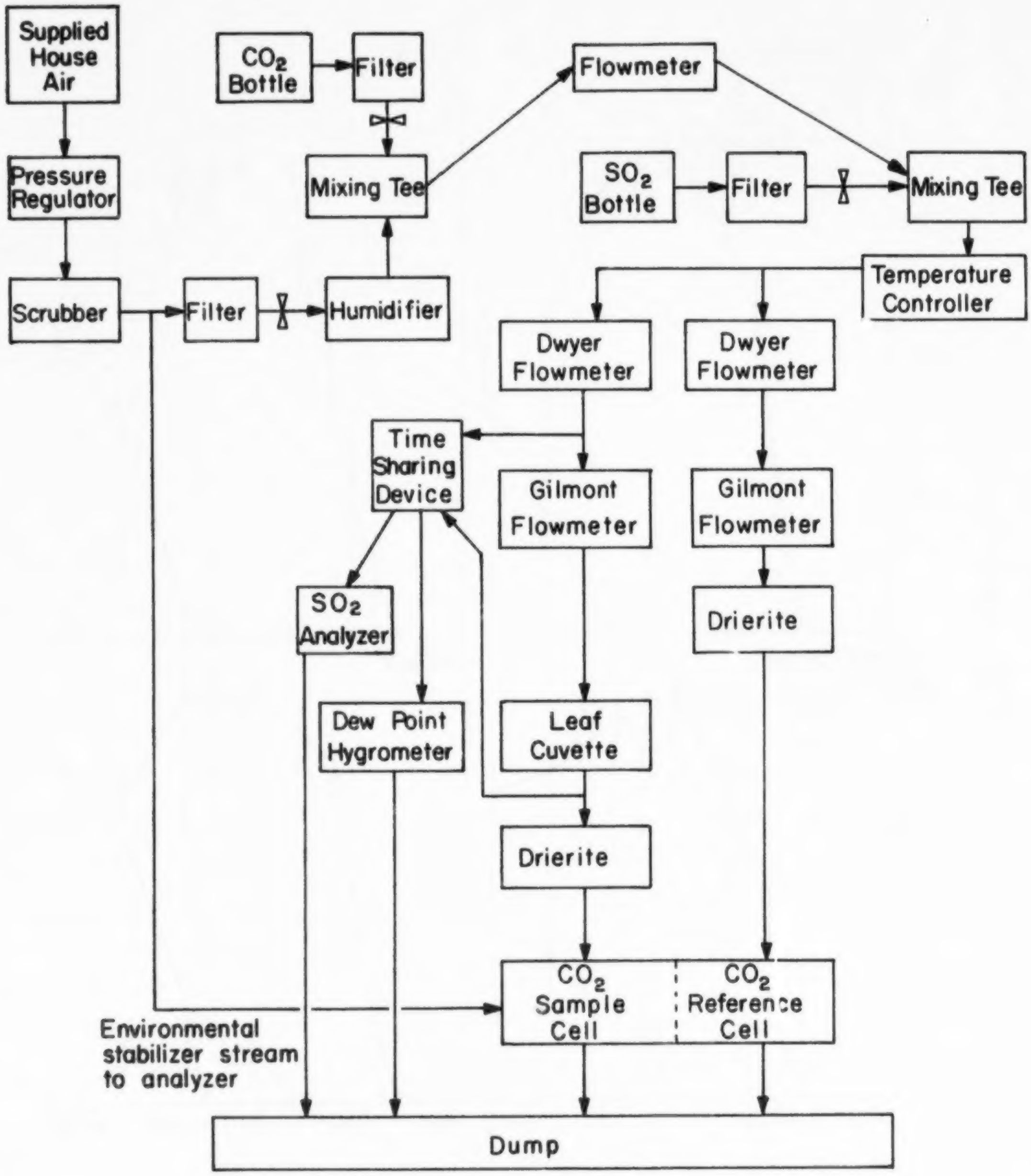

Supplied House Air
Pressure Regulator
Scrubber
CO$_2$ Bottle
Filter
Mixing Tee
Filter
Humidifier
Flowmeter
SO$_2$ Bottle
Filter
Mixing Tee
Temperature Controller
Dwyer Flowmeter
Dwyer Flowmeter
Time Sharing Device
Gilmont Flowmeter
Gilmont Flowmeter
SO$_2$ Analyzer
Drierite
Dew Point Hygrometer
Leaf Cuvette
Drierite
CO$_2$ Sample Cell
CO$_2$ Reference Cell
Environmental stabilizer stream to analyzer
Dump

Figure 13.27. Laboratory CO$_2$ exchange system.

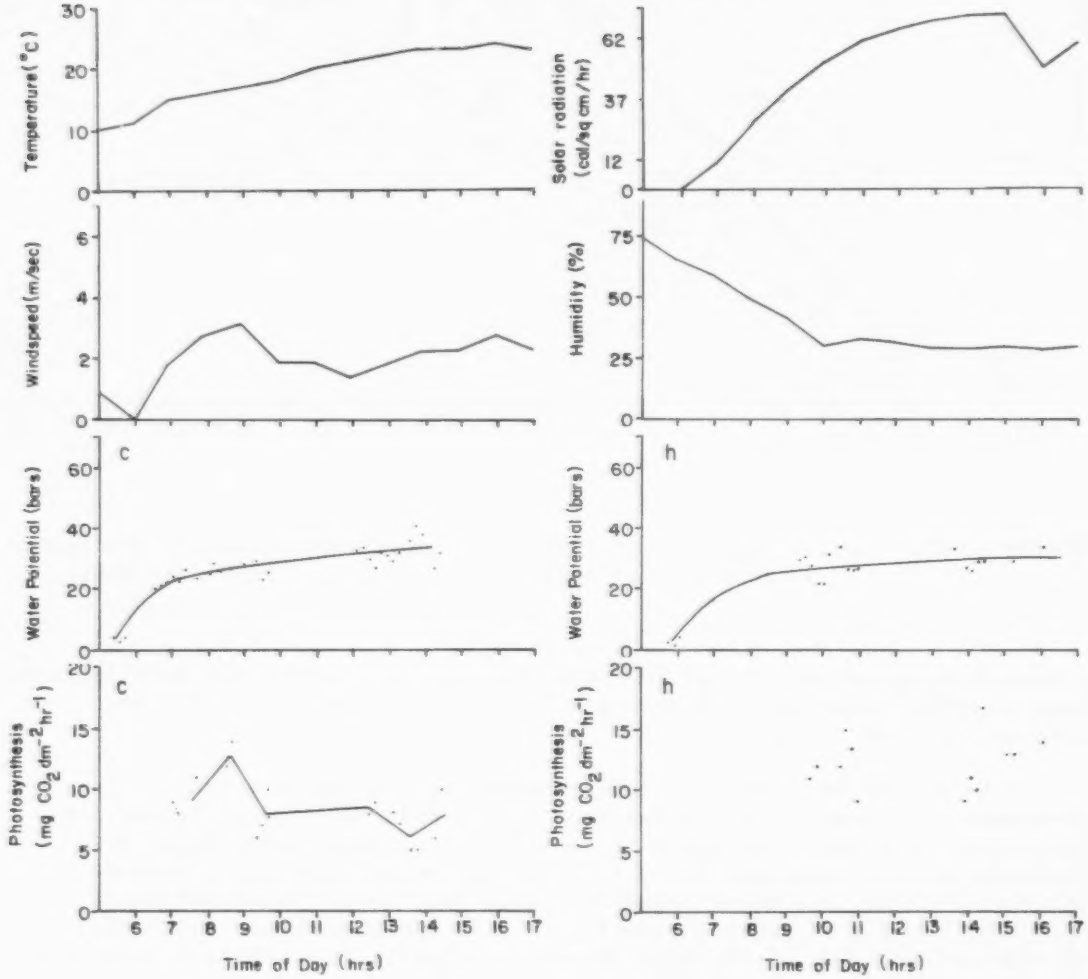


Figure 13.28. Diurnal patterns of gross photosynthesis, leaf water potential, and selected abiotic variables for western wheatgrass on the control (c) and high (h) treatments in early July 1977. (All physiological data are from observations collected during a 3-day interval; abiotic data are averages for the same 3-day period.)

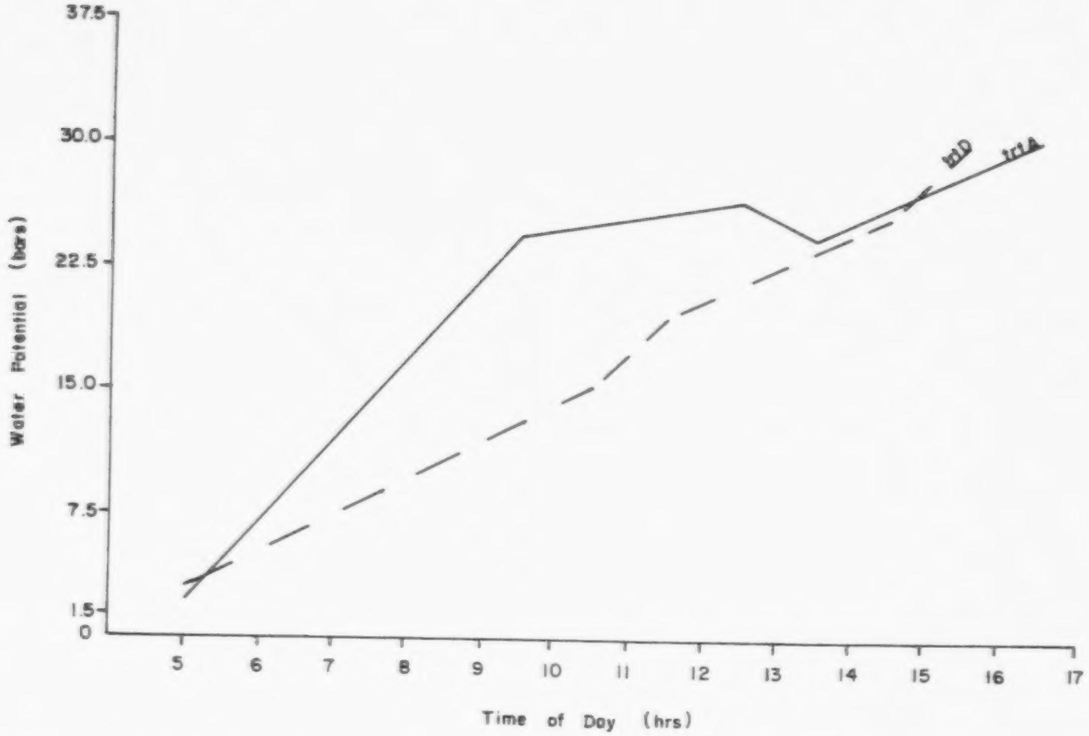


Figure 13.29. Diurnal patterns of leaf water potential in western wheatgrass for one day in July 1977 (A = control, D = high treatment).

dynamics of uptake were less clear for the high treatment. The data indicate that fixation rates may have averaged 13 mg $^{14}CO_2 \cdot dm^{-2} \cdot h^{-1}$ for the greatest portion of the days.

A more detailed view of diurnal changes in leaf water potential is presented in Figure 13.29. These data are averaged across all leaves for each plant measured. On this particular day there were substantial differences in leaf water potential between the control and high treatment plants. Predawn leaf water potentials were near zero. Water deficits developed rapidly in control plants with leaf water potential decreasing to -23 bars at 0900. Leaf water potential decreased less rapidly in plants from the high treatment and was -15 bars at 1000. Minimum leaf water potentials for plants from both areas were measured in late afternoon.

Laboratory Investigations

Our work with physiological responses of western wheatgrass to SO_2 under controlled conditions is just beginning. Figure 13.30 presents preliminary data on the response of net CO_2 exchange of western wheatgrass as a function

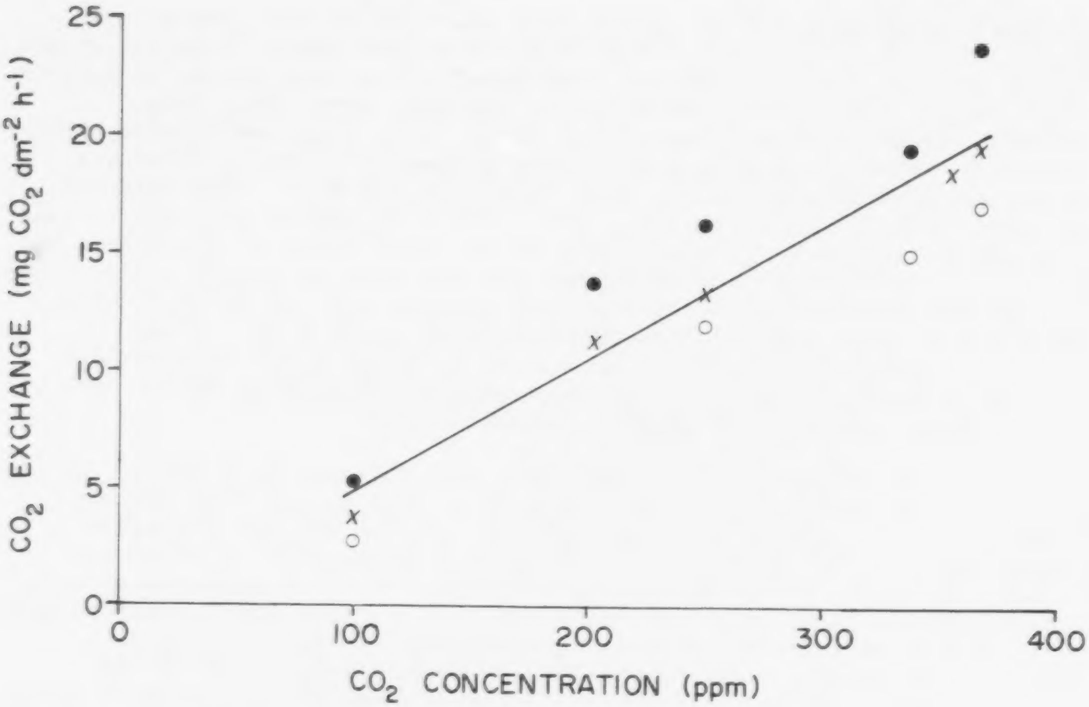


Figure 13.30. CO_2 exchange versus CO_2 concentration.

of CO_2 concentration. The three plants used in this experiment indicate a range of responses of net photosynthesis to CO_2 concentration. The regression line with a r^2 of 0.93 indicates the average for the three plants.

DISTRIBUTION OF $^{35}SO_2$ SULFUR AND $^{14}CO_2$ CARBON BY NATIVE MIXED-GRASS PRAIRIE AND POSSIBLE SO_2 EFFECTS*

Introduction

The pattern by which assimilated carbon is distributed within plants is of considerable interest to those who wish to understand fully the role played by primary producers in ecosystems. Realistic conceptual or mathematical models of ecosystems demand an understanding of both above- and belowground productivity as well as the often slighted integration of the two. A focal point for such an integration lies, of course, with the primary producers themselves, for it is they that exert the primary control over partitioning of productivity. Many researchers have looked in detail at the manner in which this distribution is governed by external influences such as light, water, temperature, and nutrients (Wardlaw, 1968, 1969, 1976; Davidson, 1969a; Lush and Evans, 1974; Barta, 1976; Evans and Wardlaw, 1976). Fewer have actually examined directly the process as it occurs in natural ecosystems (Dahlman and Kucera, 1968; Warembourg and Paul, 1973; Caldwell and Camp, 1974; Singh and Coleman, 1974), and it is indeed difficult to find adequate discussion which relates work of the prior group of researchers to the work of the latter. Little attempt has been made to integrate above- and belowground productivity in terms of both the proximal and ultimate forces operating to cause observed distributions found in the field. Plant physiologists tend to search for mechanisms at a high level of resolution while ecologists prefer to incorporate highlights of such mechanisms into the lower resolution problem of adaptive significance.

---

*Excerpted from a dissertation in preparation by Michael B. Coughenour, "Grassland Sulfur Cycle and Ecosystem Responses to Low-Level SO_2."

Roots and shoots are each involved in the exploitation of resources, but from different pools. The apportionment of assimilate and hence biomass between roots and shoots is perhaps indicative of an optimal allocation pattern by the plant to insure that above- and belowground resources are supplied in the proper proportions. This aspect of the integration of root and shoot functioning is indeed intriguing. Aboveground growth patterns have often been viewed as adaptations for maximal photosynthetic production, minimal water losses, and so forth, but are in fact constrained by the necessity that a certain proportion of photosynthate is partitioned to roots for collection of minerals and water, and structural support. If the plant has belowground perennating organs, the success of subsequent year's growth is also in the balance. In fact, non-structural carbohydrates stored by perennial grasses seem to play an important role in determining regrowth success (Weinmann, 1961; Coyne and Cook, 1970; Trlica and Cook, 1972; Bokhari, 1977).

Inorganic nutrients are also distributed and redistributed in specific patterns by plants. Over two decades ago Williams (1955) provided an extensive review on these processes. The essential feature of this work was the integration of mineral uptake and redistribution with the processes of growth and senescence to explain observations of net losses of mineral elements from specific plant parts. Thus he developed the "concept of the integrated control of the movement of nitrogen and phosphorus in the plant." In this theory nutrient uptake is determined by the external supply and the internal demand created by growth, where the external supply is more likely to be controlled by growth demands than vice versa. As each organ develops, there is at first a period of net import and accumulation of the nutrient. This is followed by a period in which import and export are balanced, and finally, during senescence export prevails. Senescing organs may than act as a nutrient source for younger organs. Uptake by roots is consequently curtailed to the extent that the nutrient is already present and reutilizable within the plant. More recently Clark's (1977) observations of internal recycling of nitrogen in shortgrass prairie have given support to Williams' ideas and re-emphasized their possible ecological significance. Short grasses were observed to remobilize considerable quantities of nitrogen from senescing tissues and transport it to belowground organs from whence it could be reutilized the following season. The adaptive significance of this behavior is that the plants conserve nutrients rather than release them to a common pool for which there is competition from other plants, microbes, and leaching losses. It also decreases the dependence of the plant on microbial mineralization from dead material.

With the advent of the industrial age, emissions of SO_2 by man-made sources have come to equal or exceed natural contributions such as fire and volcanism. Almquist (1974) estimated 80 million metric tons of SO_2 to arise annually from fossil fuel combustion and Kellogg *et al.* (1972) estimated 50 million metric tons annually. The current trend for increasing the relative contribution of fossil fuels to the national energy budget can be expected only to accentuate this situation. Ecosystems provide a considerable service to us in their capacity to remove this SO_2 from the atmosphere. It has been established that plants can derive a significant portion of their sulfur supply from the atmosphere (Alway *et al.*, 1937; Thomas *et al.*, 1943; Olsen, 1957; Ulrich *et al.*, 1967; Cowling *et al.*, 1973). In the absence of a sulfur supply to roots, plants seem capable of deriving their entire supply from this source (Faller, 1971). There is also substantial evidence for a non-metabolically active process by which live and dead leaf surfaces and soil may remove SO_2 from the atmosphere (Seim, 1970; Fowler and Unsworth, 1974; Garland *et al.*, 1974; Owers and Powell, 1974; Whelpdale and Shaw, 1974).

From such observations, the question first arises as to what the relative contribution of soil, leaf surfaces, and active processes are in this removal. A more interesting question, though, is the relationship of sulfur source to the internal distribution by the plants. Sulfur uptake and redistribution under natural circumstances may or may not have evolved under the SO_2 concentrations found today, depending on the extent of volcanism when the uptake mechanisms were evolving. Not only are normal uptake mechanisms specifically adapted to environmental substrate concentrations (Crowley, 1975) but, in addition, atmospheric substrate concentrations themselves may be under an adaptive homeostatic control by and for the biosphere (Lovelock and Margulis, 1974). This study will investigate whether or not low levels of atmospheric sulfur dioxide interfere with the adaptive mechanisms of sulfur uptake from the soil and internal redistribution by the plant.

Another aspect of SO_2 effect concerns the direct influence of this gas on photosynthesis. This subject has been treated extensively elsewhere and is not of primary concern to this study. My concern is the possible indirect effect it may have on the distribution of assimilate. This would arise if SO_2 were to affect photosynthesis but not the process of shoot growth, for the distributional fate of assimilated carbon in the plant rests on a system of priorities among organs. This is the conclusion one can draw from findings that leaves which are in active growth retain all their assimilates and import from older leaves, while older leaves export but do not import, and exports first go upward to younger leaves but only after this demand is met do they subsequently move downward to roots (Williams, 1965). It is thus conceivable, in the short term, that no changes would be observed in aboveground growth even though root growth is altered. For example, smaller reductions in leaf weight than root weight for radish under 5 pphm SO_2 have been reported (Tingey *et al.*, 1971), and ozone has been shown to reduce root growth indirectly through effects on shoot metabolism (Tingey, 1974).

Consequently, a primary objective here will be to employ a sensitive technique for in-field evaluation of photosynthate partitioning as possibly affected by low concentrations of atmospheric SO_2.

Methods

An *in situ* approach was taken to determine biomass, carbon fixation and redistribution, passive sulfur deposition on leaf and litter surfaces, active sulfur uptake, and sulfur redistribution (Figure 13.31). To evaluate temporal changes in allocation patterns, data were to be gathered throughout the growing season.

A common approach is to simply collect state-variable measurements with time (harvest method). By this method carbon allocation is deduced from ash-free biomass dynamics and sulfur allocation from total sulfur analysis. However, the precision of this method is limited by large sample variability often encountered in the field, actual flows cannot be measured directly, and measurements of live root biomass are complicated by the presence of dead roots. Another approach giving more precise estimates of flows entails the use of radio tracers. This approach, however, has limitations in accuracy when applied in a field situation, because of the practical constraints associated with small sample sizes. Large areas cannot be labeled, and the small areas which are labeled require far more extensive analysis than similar-sized samples in the harvest method.

Experimental Design and SO_2 Treatments

Experimental design consisted of two main effects, date and SO_2 treatment. Three dates in 1976 were chosen to represent early (19 May), mid (26 July), and late (5 September) growing season. The SO_2 treatment consisted of a continuous SO_2 fumigation throughout the growing season at 30-day median concentration of 10 pphm, while the control was not fumigated. The SO_2 fumigation was initiated in June of 1975, discontinued over the winter of 1975-1976, and reinitiated at the start of the 1976 growing season (April).

A detailed description of the SO_2 delivery and monitoring system can be found in Lee *et al.* (1976). Sulfur dioxide was distributed through a network of aluminum pipes positioned approximately 0.75 m above the ground. Concentration was continuously monitored by a Melloy laboratories sulfur analyzer for a 7.5-minute period every hour. Median concentration was determined daily from the total number of 7.5-minute samples. Both the fumigated and control treatments covered approximately .52 ha ground surface.

On each date, three plots were selected per treatment for labeling, giving a total of 18 plots. Four root cores were taken on each plot and divided into segments of 0-5, 5-10, and 10-20 cm. This yielded a total of 72 cores and 216 segments. Sulfur labeling was performed on the two later dates only.

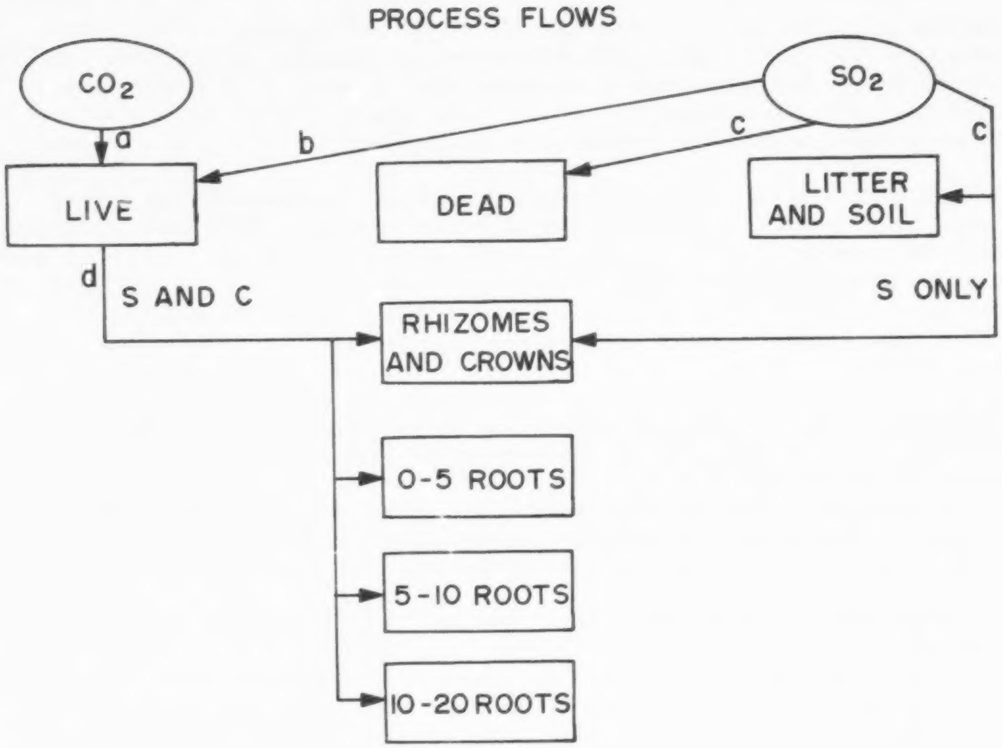


Figure 13.31. Flows measured in process study.
(a) photosynthetic carbon fixation
(b) assimilation through stomates and deposition onto live leaf surfaces
(c) deposition onto dead leaf surfaces
(d) translocation of C and S belowground

In statistical analyses, aboveground results were treated separately from belowground results. Aboveground observations were subjected to a two-way factorial analysis of variance with repeated observations. Belowground observations were subjected to a repeated-measures analysis of variance with subsampling. This method allowed for comparison between depths as well as treatments and dates and accounted for correlation substructures within core segments of the same plot. Above- and belowground treatment and date main effects were tested against a plot (date × treatment) error term. Depth effects (and interactions with depth) were tested against a depth × plot (treatment × date) error term. Means were compared using Tukey's Q test.

Labeling and Harvesting

The method used here was first implemented by Dahlman and Kucera (1968, 1969) to address specifically carbon allocation by grass plants *in situ*.

This method also was used successfully by Singh and Coleman (1973, 1974), Caldwell and Camp (1974) and refined somewhat by Warembourg and Paul (1973). Details of the method used here correspond most closely with procedures outlined by Singh and Coleman (1974). A flow chart (Figure 13.32) is presented to demonstrate the sequence of procedures.

Plots of 0.5 m^2 area were selected for visual homogeneity of cover, dominance by *A. smithii*, and presence of green biomass. Late in the season, more of the treatment cover had died, leaving only scattered patches of green material in favorable microsites. These patches were chosen for study on the grounds that location of a study plot on the mostly dead interspaces would not yield adequate information on the behavior of live material that was present.

Labeled $^{14}CO_2$ was released from a $Na_2{}^{14}CO_3$ solution inside the polyethylene tent by injection of excess $3N$ H_3PO_4 into the solution with a long-needled hypodermic syringe. A total of 450 μCi ^{14}C was released per plot. To compensate for photosynthetic CO_2 depletion, additional measured quantities of Na_2CO_3 were injected into the acidified solution. These quantities were pre-estimated to match expected net photosynthetic rates.

Labeled $^{35}SO_2$ were released simultaneously with $^{14}CO_2$ on the later two sampling dates. Sodium sulfite ($Na_2{}^{35}SO_3$) with 231 $\mu Ci \cdot g^{-1}$ (July) and 331 $\mu Ci \cdot g^{-1}$ (September) activity was measured into single plot quantities and resealed in small wide-mouthed vials. In the field, the vials were unsealed and positioned in the tents alongside the $Na_2{}^{14}CO_3$ solutions. Dropwise addition of 50% H_2SO_4 with the hypodermic needle released $^{35}SO_2$ from the salt. A total of 723 (July) and 766 μCi ^{35}S (September) was released per plot. The high specific activity of the $Na_2{}^{35}SO_3$ allowed sufficient ^{35}S to be released without introduction of significant quantities of SO_2. The concentration of SO_2 originating from the $Na_2{}^{35}SO_3$ was calculated to be only 5.3 (July) and 3.9) ng $SO_2 \cdot cm^{-3}$ (September).

The radiotracers were released in late morning, and the tents removed 5-6 hours later. No attempt was made to control temperature or humidity in the tents. By the end of the period, temperatures were several degrees higher than ambient and considerable water had condensed on the tent walls. The plots were allowed to equilibrate 5-6 days before harvesting, when all aboveground herbage was clipped from the plot.

Live material was separated by species and dead material by the categories *A. smithii* or "other." Live species represented by less than 1 $g \cdot m^{-2}$ were generally pooled as "other live." Coarse litter material was then removed by hand and fine litter by a portable vacuum cleaner. Four root cores were taken per plot with a 7.5-cm diameter coring tube to a depth of 20 cm. The cores were segmented by depth strata in the field. These root core segments were hand washed with fine jets of water over a 60-mesh screen. Material from the 0-5 cm layer was separated into roots, crowns, and rhizomes except for May in which crowns were not separated. All samples were oven-dried, weighed, ground to pass a 20-mesh screen, and mixed. Litter samples were divided into coarse and fine fractions with a 28-mesh sieve.

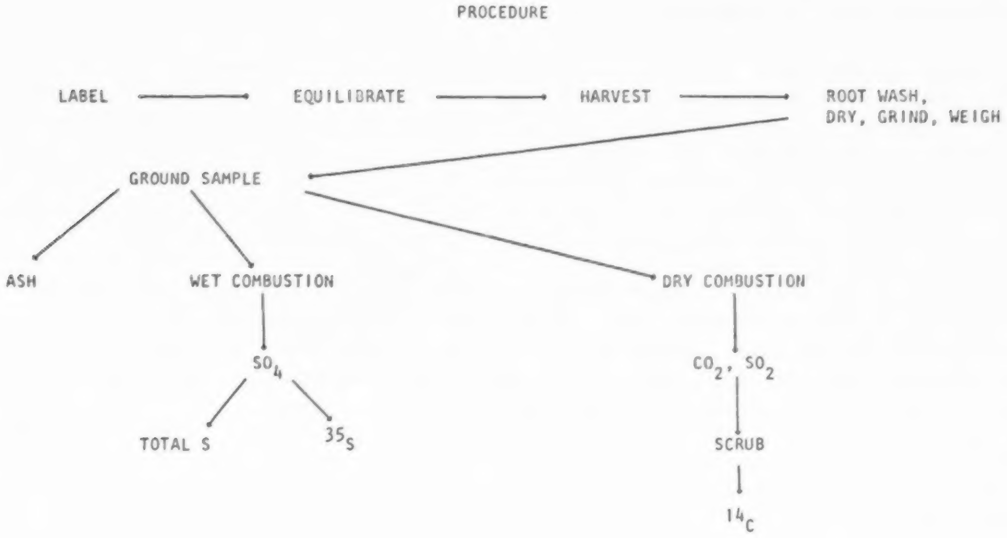


Figure 13.32. Flow chart of experimental procedures.

^{14}C Analysis

The method employed to determine ^{14}C was modified from Jeffay and Alvarez (1961a). These workers used a wet combustion technique but indicated dry combustion could be substituted. The major feature of the technique was the use of HCl solution as a gas wash to remove decomposition products other than CO_2. The method was tested (Jeffay and Alvarez, 1961b) with dually labeled (^{14}C-^{35}S) samples and ^{35}S did not interfere with the ^{14}C determination.

A 30-mg subsample was weighed into a black ashless sample wrapper suspended in a wire mesh basket within an oxygen-filled 500 ml Erlenmeyer flask. Combustion was initiated with a focused beam of infra-red light. The resulting gases were then passed in an N_2 carrier stream through two 1N HCl solutions to remove SO_2. Carbon dioxide was subsequently absorbed by 6 ml of 2:1 by volume 2-methoxyethanol and ethanolamine. Gas washing tubes were employed in the wash and absorbtion to increase contact area between gas and solution. Three milliliters of the absorbing solution was transferred to a scintillation vial with a disposable pipet. A scintillation cocktail consisting of 4.66 g $\cdot$ 1^{-1} butyl-PBD [2-(4' tent-butylphenyl)-5-(4"-biphenyl)-1,3,4-oxadiazole] in a 2:1 by volume toluene and 2-methoxyethanol was then added, and the mixture counted. Quench curves were run for ^{14}C from commercially prepared standards.

Total Sulfur and ^{35}S Analyses

Total sulfur and ^{35}S were determined on the same subsample. Total sulfur analysis was by a method modified from Stewart and Whitfield (1965). A 200-400 mg sample was weighed into a 25 × 200 mm test tube to which 3 ml of 50% $Mg(NO_3)_2$ was added. The tubes were then heated on a hot plate for 2.5 hours. Following this initial digestion, they were placed in a sand bath and ashed in a muffle furnace for 3 hours at 550°C. Ten milliliters 2N HCl was then added with slight heat and shaking. After overnight settling, 2-milliliter aliquots were taken from the supernatant and diluted to 10 ml with dionized water for turbidimetry. Barium sulfate was precipitated by addition of 0.5 g 28-48 mesh $BaCl_2$ crystals. The suspension was left standing for 60 seconds, shaken for 60 seconds, and after a total of 5 minutes, optical density was read at 495 nm. Time of standing and size of $BaCl_2$ crystals must be standardized for repeatable results (Butters and Chenery, 1959). Other workers have used dipropylene glycol rather than water during precipitation to increase the stability of the $BaSO_4$ suspension (Krober and Howell, 1958). Orthophosphoric acid may also be added to decolorize iron in soil or root samples (Butters and Chenery, 1959).

The $BaSO_4$ suspension was combined with the remainder of the original supernatant in a scintillation vial, and an additional 0.5 g $BaCl_2$ crystals were added. The combined $BaSO_4$ suspension was then centrifuged at 1800 rpm for 15 minutes. A water aspirator equipped with a hypodermic needle was used to draw off the supernatant. The precipitate was counted after suspension in 600 mg Cab-O-Sil gel with 15.0 ml of Brays' dioxane-based cocktail (Bray, 1960).

Secondary ^{35}S standards were prepared for determination of quench curves. A ^{14}C primary standard and the unknown ^{35}S solution were counted with a GM device under various absorber thicknesses. By knowing the ^{14}C true activity, one could then regress count rate against absorber thickness, extrapolate to zero thickness, and thus determine the efficiency of the GM device. Because ^{35}S and ^{14}C have very similar energy spectra, this efficiency can be used to calculate the true ^{35}S activity at the extrapolated zero absorber thickness for the ^{35}S solution. A quenching agent (CCl_4) was then added to the standardized solutions in varying amounts.

A test sample containing only ^{14}C label was subjected to this procedure to determine whether ^{14}C interfered with the ^{35}S counts. A small amount of ^{14}C contamination was present even after the $Mg(NO_3)_2$ oxidation. To account for this contamination, samples were recounted after a period of radioactive decay. Because ^{14}C has a half life (5730 yr) which is very large relative to that of ^{35}S (88 days), any radioactive decay after the interval between counts could be ascribed to ^{35}S. The basic decay equation can be manipulated to give the desired result: letting N_1 equal count rate on first date, N_2 equal count rate second date, and t_i equal time interval between count dates, the manipulation is:

$$N_2/N_1 = e^{-\lambda t_i} \quad (1)$$

$$(1 - N_2/N_1) = (1 - e^{-\lambda t_i}) \quad (2)$$

$$(N_1 - N_2) = N_1(1 - e^{-\lambda t_i}) \quad (3)$$

Letting the measured decay over t_i be $\Delta = N_1 - N_2$, and replacing N_1 with N_1* which represents the true ^{35}S count rate on the first date, then:

$$N_1* = \frac{\Delta}{(1 - e^{-\lambda t_i})} \quad (4)$$

Activity at time of manufacturer standardization can be calculated as:

$$N_o = N_1*/e^{-\lambda t_o} \quad (5)$$

where t_o = time interval between standardization and first count date. Time interval between counts was between 0.5 and 1.0 half life of ^{35}S.

General

Both isotopes could be counted under the same window settings because of their energy similar spectra. The external standard-channels ratio technique was used to determine counter efficiency. Counting time was 1-20 minutes depending on sample activity.

Raw data for biomass, ash, and count rates was processed by computer to yield ash-free biomass, flux rates $\cdot$ g^{-1} and flux rate $\cdot$ m^{-2} ground surface. Because SO_2 uptake and deposition is proportional to atmospheric SO_2 concentration (Hill, 1971), flux rates for sulfur were normalized with respect to concentration. This facilitated comparisons between the two dates in which different $^{35}SO_2$ concentrations were used. The resulting quantities are referred to as deposition velocities (Vg), where Vg = F/C. If concentration (C) is given in $\mu g\ S \cdot cm^{-3}$ and flux rate (F) is given in $\mu g\ S \cdot g^{-1}$ biomass $\cdot\ h^{-1}$, Vg takes the units of $cm^3 \cdot g^{-1}$ biomass $\cdot\ h^{-1}$. When F is given in $\mu g\ S \cdot m^{-2}$ ground $\cdot\ h^{-1}$, Vg takes the units of $cm^3 \cdot m^{-2}$ ground $\cdot\ h^{-1}$ which is then converted to $cm \cdot sec^{-1}$.

Aboveground results viewed in terms of flux per gram of biomass can readily be converted to a flux per unit leaf area as

$$\frac{F'}{LA} = \frac{F'}{DWT} \cdot \frac{DWT}{LA} \quad (6)$$

where F' is absolute flux (μg SO_2), LA is leaf area (cm^2), and DWT is dry weight. Flux per unit ground area also reflects the magnitude of aboveground biomass as

$$\frac{F'}{GA} = \frac{F'}{DWT} \cdot \frac{DWT}{GA} \tag{7}$$

where GA is ground area (m^{-2}).

Count rates of ^{35}S were scaled so that the mean plot total deposition velocity for control treatments in July and September agreed with Owers and Powell's (1974) mean Vg for grassland of 0.7 cm · sec^{-1}. Thus, all count rates were multiplied by the same scaling factor. Reasons for scaling include: (a) actual $^{35}SO_2$ concentrations were not monitored in the tents, (b) large amounts of $^{35}SO_2$ were probably absorbed on tent walls, (c) not all of the $^{35}SO_2$ was released from the salt, and (d) the primary intent of this study was to make relative comparisons between units of the study rather than to measure deposition rates absolutely. The value of Vg = 0.7 seems appropriate because (a) it was determined for a grassland, (b) it was measured on a ground rather than leaf area basis, (c) measurements were under similar climatic conditions as would be found here, (d) sulfur deposited on stem bases and ground surface was accounted for, (e) reference concentration height was 20 cm, similar to tent height, and (f) the tracer method used for its estimation is a more direct approach than inference from concentration profiles.

As for ^{35}S, ^{14}C count rates were scaled. This was found to be necessary because abiotic conditions in the tents were not representative of real ambient conditions and $^{14}CO_2$ concentrations in the tent were not monitored. The scale was chosen so that aboveground relative growth rates in May for *Agropyron smithii* agreed with field observations for the period mid-May to mid-June of 1.07 mg C · g^{-1} biomass C · h^{-1} (Heitschmidt, 1977). As a result of scaling, comparisons of absolute values must be confined within this study.

Results

$^{14}CO_2$ Assimilation and Translocation

Aboveground growth rates were significantly higher in May than other dates for *Agropyron smithii* (Table 13.16). The other species collectively maintained high relative growth rates throughout the season; however, their contribution to total aboveground biomass declined, leaving absolute growth increments dependent mostly on *A. smithii*. For total live material, the growth rate was significantly different between all dates with a progressive drop-off throughout the season. This decline was greater from May to July than from July to September. No significant treatment effects or interactions were noted for aboveground growth.

TABLE 13.16. RELATIVE AND ABSOLUTE GROWTH RATES (^{14}C COUNTS ARE SCALED)

Species or plant parts	May				July				September			
	Control		10 pphm		Control		10 pphm		Control		10 pphm	
	$mg\ C \cdot g^{-1} \cdot hr^{-1}$	$mg\ C \cdot m^{-2} \cdot hr^{-1}$	$mg\ C \cdot g^{-1} \cdot hr^{-1}$	$mg\ C \cdot m^{-2} \cdot hr^{-1}$	$mg\ C \cdot g^{-1} \cdot hr^{-1}$	$mg\ C \cdot m^{-2} \cdot hr^{-1}$	$mg\ C \cdot g^{-1} \cdot hr^{-1}$	$mg\ C \cdot m^{-2} \cdot hr^{-1}$	$mg\ C \cdot g^{-1} \cdot hr^{-1}$	$mg\ C \cdot m^{-2} \cdot hr^{-1}$	$mg\ C \cdot g^{-1} \cdot hr^{-1}$	$mg\ C \cdot m^{-2} \cdot hr^{-1}$
Achillea millifolium	0.151	0.013	0.239	1.37	0.316	2.78			2.486	21.72	0.097	0.985
Agropyron smithii	0.452	20.93	0.402	20.34	0.154	21.76	0.214	26.19	0.113	11.84	0.159	26.52
Antennaria rosea							0.126	1.32	0.058	1.19		
Aristida longiseta							0.164	1.08				
Artemisia frigida							0.245	0.61				
Bouteloua gracilis									0.167	0.32		
Bromus japonicus	0.133	0.120	0.276	0.196								
Koeleria cristata	0.264	7.53	0.233	1.91	0.250	8.46	0.444	2.73	0.180	0.888		
Poa pratensis									0.027	0.313		
Poa secunda	0.974	12.07	0.207	1.01			0.345	13.10				
Stipa comata	0.354	2.26			0.180	1.78	0.238	0.712				
Stipa viridula			0.318	0.381								
Taraxacum officinale			0.262	0.389	0.461	0.477	0.547	1.11				
Tragopogon dubius	0.198	0.341	0.347	1.59	0.348	1.57	0.005	1.80				
Other	0.348	2.33	0.209	1.50					0.173	1.48	0.221	2.27
Total	0.441	43.61	0.340	29.08	0.395	23.82	0.270	47.00	0.247	16.76	0.198	28.87
Rhizomes	0.031	1.54	-0.014	0.643	0.018	1.00	0.031	0.40	0.009	0.176	0.004	0.396
Crowns	--	--	--	--	0.019	1.24	0.029	3.58	0.009	1.03	0.009	1.07
0-5 cm roots & crowns	0.036	26.8	0.329	18.38	0.029	18.23	0.062	23.98	0.004	4.41	0.004	2.49
0-5 cm roots	--	--	--	--	0.033	17.01	0.063	22.41	0.003	1.19	0.003	1.42
5-10 cm roots	0.019	2.73	0.026	1.39	0.030	4.00	0.017	7.02	0.042	1.41	0.033	0.565
10-20 cm roots	0.024	2.81	0.031	4.37	0.011	4.20	0.014	1.08	0.009	1.17	0.004	0.455

Taken over all depths and treatments, the highest relative root growth rates were in July (Figure 13.33). Roots in the 0-5 cm layer grew at a faster rate in May and July than in September, while below 5 cm, roots grew fastest in July. A significant treatment × date interaction (P = 0.032) was found because the treatment effect was noted only in July. At this time, aboveground biomass was lower on fumigated plots (Table 13.17) yet root growth rates were higher. There was neither a significant depth × treatment interaction (P = 0.06) nor a significant three-way interaction of depth × date × treatment (P = 0.093). The significant treatment × date interaction seemed attributable to July, when 5-10 and 10-20 cm roots were growing at a faster rate in the SO_2 treatment.

The percentage of total ^{14}C found below ground (Figure 13.34) declined from 45.27% in May and 51.46% in July to 17.04% in September. Strictly, total translocation below ground would also have to include root respiration, exudation, and sloughing. For example, Warembourg and Paul (1973), working on a similar floristic composition grassland, found roots respired 9% to 15% of the total plant assimilate, or 20% to 29% of the total translocate. Singh and Coleman (1977) found shortgrass prairie roots to respire 9% to 19% of total plant assimilate. Therefore, the partitioning of carbon observed here represents the relative increments in biomass (here referred to rather loosely as "growth") between roots and shoots or between depths rather than the allocation of total assimilate. Also, if these figures were adjusted on the assumption that 30% of the roots were actually found below 20 cm, the proportion of total growth occurring belowground would be 51% in May, 57% in July, and 22% in September.

A greater percentage of total belowground growth was found in deeper depths later in the season (Figure 13.34). A greater percentage was also found in rhizomes as the season progressed, and between July and September crowns also received an increased percentage. These results agree with those of Singh and Coleman (1973) who found maximum growth in the 0-10 cm depth from late May to July and maximum 10-20 cm growth from late July to September. The results, however, conflict with their finding that relative allocation to crowns decreased with time. The findings here correspond to those of Warembourg and Paul (1973), who found decreased percentages in 0-10 cm roots in late season and in 10-25 cm roots in mid-season.

$^{35}SO_2$ Fluxes and Distributions

Certain species were notably more active in SO_2 uptake than others (Table 13.18). *Achillea millefolium* and *Taraxicum officinale* ranked highest in dry weight basis SO_2 assimilation rates. *Antennaria rosea, Aristida longiseta*, and *Artemisia frigida* were among the lowest. There was a date effect for *Agropyron smithii* (P = 0.046) with less uptake in July as well as a treatment effect (P = 0.02) with less uptake on the control. For total live material, no significant treatment effect was observed (P = 0.08), but the trend was for less uptake on the control. *Tragopogon dubius* and *Taraxium officinale* seemed especially affected by treatment, and while statistical analyses were not performed for these species, uptake appeared lower in the control. On the other hand, *Koeleria cristata* exhibited higher

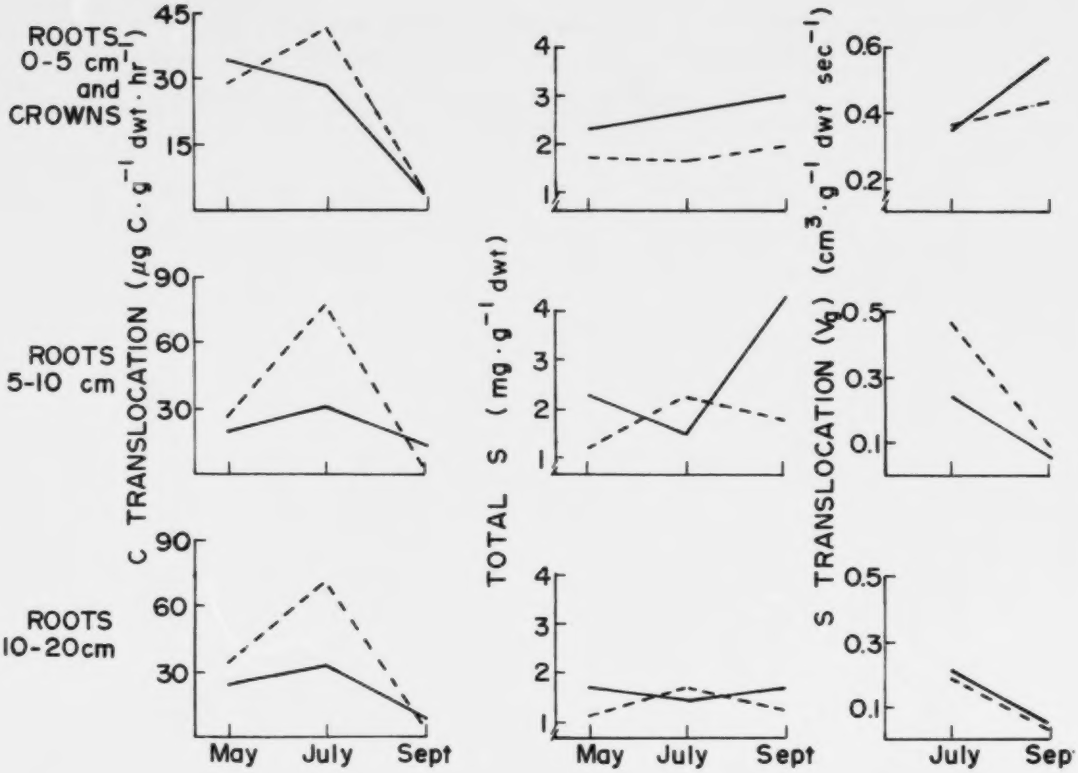


Figure 13.33. Belowground ^{14}C net translocation per hour, total sulfur, and ^{35}S net translocation. Solid lines refer to control. Dashed lines refer to continously fumigated 10 pphm treatments.

uptake on the control. Dry deposition onto dead material was not affected by date or treatment. The difference between deposition rates on *Agropyron smithii* live and dead materials was more pronounced on fumigated treatments. Deposition onto litter material was not affected by date or treatment.

A significant depth (P = 0.0001) and depth by date interaction (P = 0.0019) was found for translocation of ^{35}S to roots (Figure 13.33). Significantly more sulfur was translocated on a dry weight basis to 0-5 cm roots in September than in July, but significantly less was translocated to 5-10 and 10-20 cm roots in September than in July. Depths behaved similarly in July, but in September 5-10 and 10-20 cm roots received less than crowns or 0-5 cm roots. The increase in translocation to the 0-5 cm layer from July to September indicates that this layer became a more efficient sulfur sink as the season progressed. The opposite was true at 5-20 cm.

Although treatments behaved differently with respect to sulfur distribution on a ground area basis (Figure 13.35), they are lumped as one since treatment differences in biomass, which affect these distributions (see

TABLE 13.17. STANDING CROP OF PLANT MATERIALS ON LABELLED TEST PLOTS, 1976*

Species	May		July		September	
	Control	10 pphm	Control	10 pphm	Control	10 pphm
Live						
Agropyron smithii	46.3	51.1	167.3*	113.5*	103.4	133.3
Koeleria cristata	28.6*	8.2*	36.8*	6.1*	6.2*	0.0*
Stipa viridula	0.0	1.2	0.0	0.0	0.0	0.0
Stipa comata	6.4	0.0	7.1	2.8	0.0	0.0
Poa pratensis	0.0	0.0	0.0	0.0	11.6	0.0
Poa secunda	12.4	4.9	0.0	24.0	0.0	0.0
Tragopogon dubius	1.7	4.6	4.5	3.6	0.0	0.8
Achillea millefolium	0.1	5.3	8.8	0.0	8.9	1.5
Artemisia frigida	0.0	0.0	0.0	3.7	0.0	0.0
Antennaria rosea	0.0	0.0	0.0	12.1	20.7	0.0
Bromus japonicus	0.9	1.4	0.0	0.0	0.0	0.0
Taraxicum officinale	0.0	1.5	0.7	2.4	0.0	0.0
Aristida longiseta	0.0	0.0	0.0	1.4	0.0	0.0
Bouteloua gracilis	0.0	0.0	0.0	0.0	1.7	0.0
Other	6.7	7.2	0.0	0.0	8.5	10.1
Total	103.3	85.4	225.2	169.6	161.2	145.8
Dead						
Agropyron smithii	75.5	77.3	88.9	65.5	63.7	74.8
Other	44.9	25.7	83.3	83.3	25.3	17.5
Total	120.4	103.0	172.2	148.8	89.0	92.3
Litter						
Coarse	108.8*	171.9*	99.5*	211.8*	164.3*	215.4*
Fine	17.9	19.7	71.2	115.0	74.4	85.3
Total	126.7*	191.5*	170.7*	327.8*	238.7*	300.7*
Belowground						
Rhizomes	43.88	43.26	72.89	66.53	95.67	69.92
Crowns	--	--	106.45	102.61	128.30	98.32
0-5 cm roots + crowns	810.81*	657.01*	670.20	646.27	1118.22*	576.95*
0-5 cm roots	--	--	563.74	543.65	989.91*	478.62*
5-10 cm roots	140.03	130.17	108.40	95.60	230.79*	103.75*
10-20 cm roots	117.58	127.70	120.39	102.11	212.98*	103.97*

*Indicates treatment difference (g · m^{-2}).

Equation 7), were present. On all dates the greatest quantities were in or on aboveground herbage with 39% in July and 54% in September. Litter material received the next greatest proportion with 33% in July and 29% in September. Dead aboveground material received 24% in July and 11% in September, while belowground materials received only 4% in July and 7% in September. The percentage of sulfur-35 in all harvested live material (above and belowground) that was translocated belowground ranged from 8.0% on fumigated plots to 14.8% on control plots in September. Seasonal trends were reversed with a seasonal decrease from 9% to 1.8% on the control and an increase from 2.9% to 14.8% on the fumigated plots. These figures compare with 5.2% found in aspen to 24.3% in sugar maple seedlings after 8 days (Jensen and Kozlowski, 1975) and a range of 1.4% to 2.6% for tobacco (Faller, 1971).

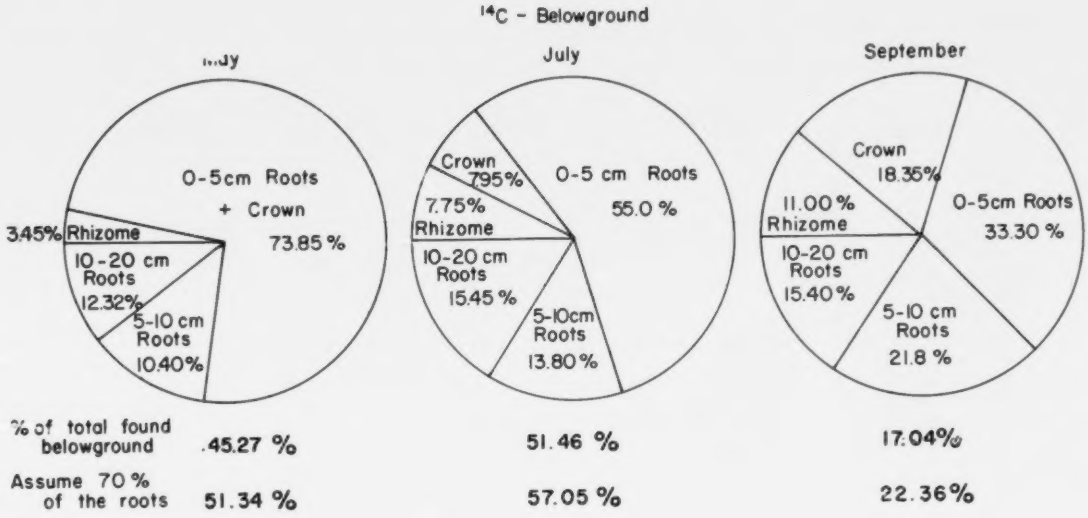


Figure 13.34. Distribution of ^{14}C belowground assimilate on ground area basis.

Distribution among rooting depths and organs was unaffected by treatment (Figure 13.36). A highly significant depth × date interaction ($P \simeq 0$) indicated both the overwhelming importance of 0-5 cm roots and the apparent increase in 0-5 cm sink strength as the season progressed, with 66% in July and 80% in September found there. Translocation to crowns seemed unaffected by date, with an average of 11.3% being translocated there, or to rhizomes with 4.7% sent there on both dates. Roots below 5 cm received a smaller proportion with time with 17.9% in July and 4.8% in September.

Total Sulfur

Total sulfur (Table 13.19) was significantly higher on the fumigated treatment for *Agropyron smithii* ($P = 0.003$) and for the rest of the live material taken as a group ($P = 0.0001$). Sulfur content of coarse litter increased with time on the control treatment but decreased with time on fumigated plots. Sulfur content of fine litter increased between May and July on both treatments and declined little in September. Levels were significantly higher under fumigation for coarse litter only in May, while in fine litter, the treatment effects were apparent at all dates ($P = 0.024$).

Belowground results (Figure 13.33) were somewhat unexpected. Taken over all dates and treatments, there were significantly greater sulfur contents in roots on the control treatment ($P = 0.05$). The only exceptions to this were in July below 5 cm, but these were not of sufficient magnitude to cause significant two or three-way interactions. A significant depth effect ($P = 0.036$) was found with rhizomes and 10-20 cm roots having lower values than 0-5 cm roots plus crowns or 5-10 cm roots. On the control plots, the data

TABLE 13.18. RELATIVE SO_2 DEPOSITION VELOCITIES*

Species	July				September			
	Control		10 pphm		Control		10 pphm	
	DWT	GA	DWT	GA	DWT	GA	DWT	GA
Live								
Achillea millifolium	20.32	0.027			30.61	0.027	32.47	0.005
Agropyron smithii	7.72	0.129	19.74	0.224	18.31	0.189	22.74	0.303
Antennaria rosea			3.58	0.004	6.15	0.013		
Aristida longiseta			2.86	0.0004				
Artemisia frigida			3.72	0.001				
Bouteloua gracilis					19.03	0.003		
Koeleria cristata	31.19	0.115	16.45	0.01	11.73	0.007		
Poa pratensis					30.05	0.035		
Poa secunda			41.78			0.100		
Stipa comata	11.73	0.008	11.87	0.003				
Taraxicum officianale	50.22	0.003	77.98	0.018				
Tragopogon dubius	16.88	0.007	38.63	0.014				
Other					20.17	0.017	35.48	0.036
Total	13.45	0.289	23.17	0.274	17.60	0.391	23.61	0.344
Dead								
Agropyron smithii	7.15	0.063	9.44	0.061	8.44	0.54	6.43	0.048
Other	10.73	0.089	17.59	0.146	9.30	0.023	13.45	0.023
Total	8.87	0.152	14.16	0.207	8.73	0.077	8.16	0.071
Litter								
Coarse		0.082		0.223		0.100		0.071
Fine		0.074		0.144		0.122		0.100
Total		0.156		0.367		0.222		0.171
Rhizome		0.0013		0.0015		0.0019		0.0019
Crown		0.0035		0.0036		0.0073		0.0032
Total belowground plant material (+ roots)		0.029		0.031		0.068		0.030
Above and belowground total		0.626		0.879		0.758		0.616

*^{35}S counts are scaled so mean of July and September control plots total V_s = 0.7 cm · sec^{-1}. DWT = dry weight basis (cm^3 · C DWT^{-1} · h^{-1}), GA = ground area basis (cm · sec^{-1}).

suggested a draw-down in sulfur concentration below 5 cm between May and July but not at 0-5 cm. Between July and September, all layers on control plots showed an accumulation of sulfur. On the fumigated treatments, 5-10 and 10-20 cm depths accumulated sulfur from May to July, and there was decrease from July to September, a marked contrast to control plots. Total sulfur in the 0-5 cm layer appeared consistently greater on control plots at all dates and increased with time on both treatments.

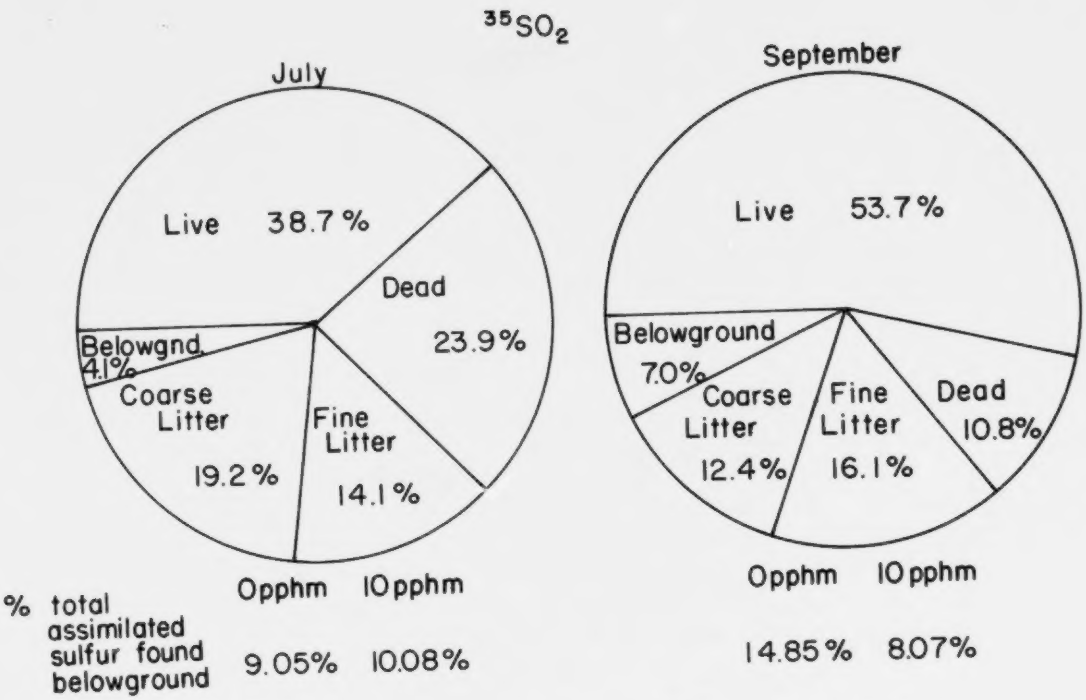


Figure 13.35. Distribution of $^{35}SO_2$-sulfur for entire labeled plots.

Discussion

$^{14}CO_2$ Assimilation and Distribution

Soil water has often been thought to be a primary determinant of relative root to shoot growth, but such reports are often conflicting. Some findings indicate that drought stress favors root growth relative to shoot growth (Davidson, 1969b, Evans and Wardlaw, 1976). Although translocation to roots is not, in the strictest sense, identical to root growth (because not all the translocate may be incorporated into live tissue), the quantities of assimilate translocated to roots is useful as a general indicator of relative root and shoot growths. With this qualification, the finding of 10% more net translocation belowground in July (when 0-30 cm soil water was less) than in May is of interest (Singh and Coleman, 1973). On the other hand, *Agropyron cristatum* translocated greater percentages belowground under wet conditions (Sosebee and Wiebe, 1971) and root growth was suppressed under water stress in *Lolium temulentum* (Wardlaw, 1969).

Total soil water in the top 105 cm of soil averaged over the entire treatment areas was increasing from 14 cm in May and decreasing from 24 cm in July, with lowest levels in September (9 cm). All labeling dates had soil water contents below the maximum observed of 24 cm on 21 June (Section 11,

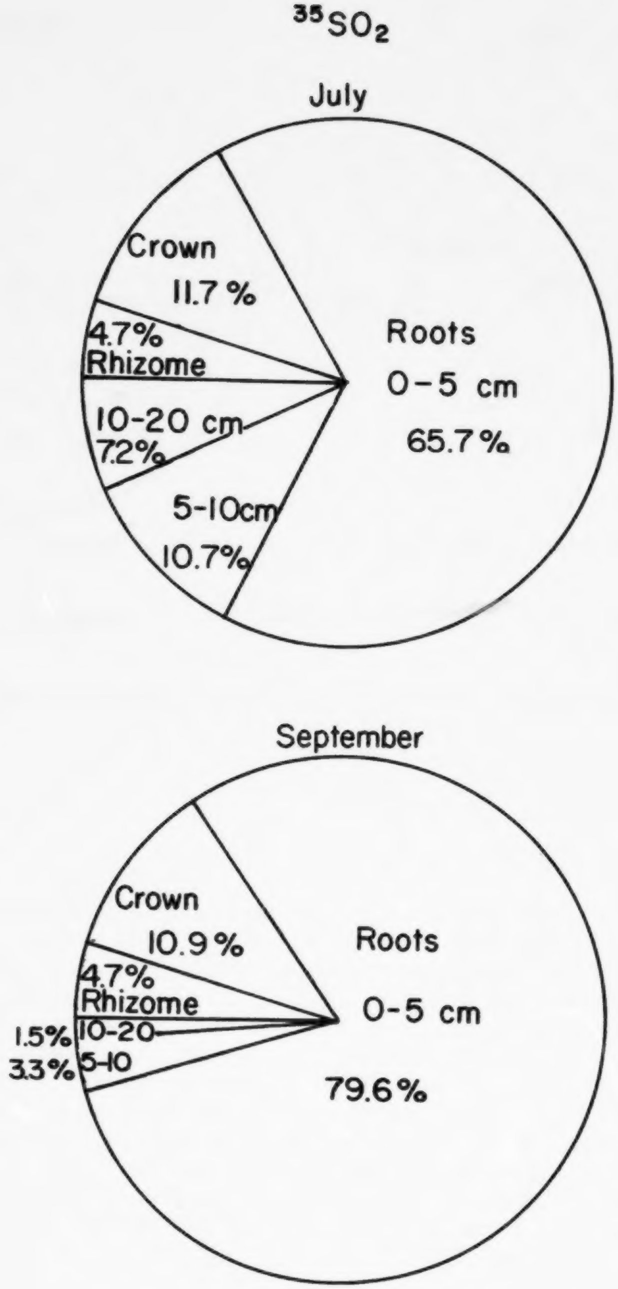


Figure 13.36. Belowground distribution of $^{35}SO_2$-sulfur.

TABLE 13.19. TOTAL SULFUR ANALYSES*

Species	May				July				September			
	Control		10 pphm		Control		10 pphm		Control		10 pphm	
	mg S/g	mg S/m^2	mg S/g	mg S/m^2	mg S/g	mg S/m^2	mg S/g	mg S/m^2	mg S/g	mg S/m^2	mg S/g	mg S/m^2
Live												
Agropyron smithii	0.7	33.34	2.4	124.63	0.7	117.10	3.4	385.90	0.7	72.40	3.9	519.90
Koeleria cristata	3.9	107.84	3.9	34.63	3.2	121.64	5.1	28.70	3.2	111.21		
Stipa viridula			5.9	5.88								
Stipa comata	2.1	13.71			3.1	23.16	4.4	12.55				
Poa pratensis									4.7	43.10		
Poa secunda	3.9	49.43	4.0	26.74			8.2	198.63				
Tragopogon dubius	4.8	8.23	7.6	34.72	7.0	27.43	9.4	37.73				
Achillea millifolium	4.4	0.40	4.4	22.43	5.9	52.37			1.3	11.25	4.1	3.94
Artemisia frigida							2.6	9.61				
Antennaria rosea							5.5	66.39	1.0	21.34		
Bromus japonicus	4.5	4.27	5.5	7.70								
Taraxicum officinale			5.4	8.07	8.5	6.36	12.2	28.72				
Aristida longiseta							4.4	6.20				
Bouteloua gracilis									2.0	3.55		
Other	4.2	34.27	6.6	50.17					2.7	14.50	5.6	51.97
Total		245.50		314.86		348.02		774.43		277.35		575.80
Dead												
Agropyron smithii	0.7	50.23	1.0	76.10	0.7	62.20	1.2	78.60	0.7	44.60	2.0	149.60
Other	0.8	32.24	1.9	52.28	0.2	173.60	5.2	444.40	0.7	17.74	1.7	29.39
Total		82.47		128.38		255.80		523.00		62.30		180.00
Litter												
Coarse	0.7	82.04	4.3	703.32	1.1	109.08	2.8	546.82	1.5	241.03	1.4	291.71
Fine	1.8	30.52	4.3	77.25	3.9	263.40	7.0	774.80	3.9	286.60	6.3	525.80
Total		112.60		780.60		327.50		1321.60		527.60		817.50
Rhizomes	2.1	22.90	2.0	21.60	1.2	21.80	1.9	31.50	1.2	28.70	1.0	17.50
Crowns					1.3	138.30	1.4	143.60	1.4	179.60	1.1	108.10

***Agropyron smithii* analyzed by Leco Induction Furnace method.

Figure 11.1). Thus, soil water was somewhat low on all dates but more so in September. Location of plots on green patches in September probably biased soil water such that labeled plots in September were wetter than the entire treatment areas in which soil water was estimated. Thus, soil water on my study plots was likely similar on all dates.

The translocation system is insensitive to water stress, but translocation can be altered by water stress indirectly through effects on growth (Wardlaw, 1968). This hypothesis taken with observations that growing shoots have higher priority over assimilate than roots (Williams, 1965) would lend support to findings that growth belowground is more limited by water stress than that aboveground. Since there were no significant differences in absolute growth rates aboveground between dates (Table 13.16) and soil water on the study plots (versus that on entire treatment areas) was similar on all dates, soil water was probably not a proximal cause for these distributions.

Translocation also may be related to the phenology of the plant. *Phleum pratense* translocated less to roots during ear emergence than at stem growth initiation (Balasko and Smith, 1973). *Lolium perenne* under continuously favorable soil water translocated about 50% to roots in the 4-leaf stage but less than 10% in the 13-leaf stage (Ryle, 1970a). On the other hand, the proportion of photoassimilate found in shortgrass prairie roots increased with phenological stage (Singh and Coleman, 1977).

Temperature conditions also may play a role. High temperature pretreatment reduced the percentage export of ^{14}C from barley leaves (C3 species) but increased export from sorghum leaves (C4 species) (Wardlaw, 1976). In the 1976 review, Evans and Wardlaw concluded that low temperatures tended to favor root growth relative to shoot growth. Davidson (1969a) established that under constant air temperature, an increase or decrease in soil temperature from 20°C favored higher root to shoot ratios in *Lolium perenne* and *Poa pratensis*. Here, air temperatures in the tents during labeling were 25-30°C in May, 28-30°C in July, and 29-32°C in September, so no real differences between dates were likely. Soil temperatures most likely continued to increase throughout the season.

Lowered light regimes seem to decrease translocation to roots (Ryle, 1970b, Lush and Evans, 1974, Ryle and Powell, 1976). Also, Mitchell (1954) found assimilate in ryegrass to be partitioned to areas of growth nearest to the source of the assimilate when plants were shaded. Incident light was probably nearly equal in May and July but lower in September. I suggest that the higher soil temperatures and the lower available light, in view of the above findings, might support a lowered root growth relative to shoot growth late in the season as observed. These findings also agree with direct observations of root growth by Ares (1976) in a shortgrass prairie where root growth was maximum when leaf expansion was greatest and soil water highest.

The partitioning of total translocate among plant parts and root layers (Figure 13.34) seem best explained in terms of soil water and the role of 0-5 cm roots, crowns, and rhizomes as storage organs for early spring growth. In early parts of the season, the optimal strategy would be to partition a large quantity of translocate to new roots at shallow depths in order to establish a functional root biomass that, in the later parts of the season (when the top of the profile is dry), can intercept rain immediately. In terms of competition between plants, species which as individuals are able to utilize mid- and late-season rain most efficiently would have a selective advantage. Such plants would require an adequate concentration of roots higher in the profile. This is, perhaps, the explanation for the tremendous concentration of root biomass in the 0-5 cm layer (Table 13.17). Later in the season during dry-down of the top soil layers, the advantage shifts to deeper depths, where the conditions for root growth are still favorable. Increased percentages going to storage organs, such as crowns and rhizomes, in late season may be part of a strategy to maintain high reserves over winter and thus gain a competitive advantage during growth initiation in the spring.

Treatment Effects on Root Growth

Treatment effects on root growth below 5 cm in July may have been an artifact of species composition differences in rooting patterns, a definite possibility because of the greater abundance of *Koeleria cristata* on control plots. To test for a correlation of root growth rate at the 5-10 and 10-20 cm depths with species composition, an analysis of covariance model was constructed with two covariates: the ratio of biomass and the ratio of ^{14}C assimilation per square meter between *Agropyron smithii* and *K. cristata*. The covariates together were related to a highly significant portion of the variation at both the 5-10 cm depth ($P = 0.003$) and at the 10-20 cm depth ($P = 0.001$). Even after this significant adjustment for species composition had been made, somewhat significant treatment effects persisted at both depths ($P = 0.10$, $P = 0.06$). Care must be taken in interpretation of these results, for it is possible for the response to be correlated with species composition yet that causality not be present.

The observed response could have been caused by the following: (a) *K. cristata* has lower root growth at these depths (b) *A. smithii* has lower root growth at these depths (c) *Poa pratensis* has greater root growth at these depths. If (a) were true, it seems likely that a greater root biomass would have been observed on the fumigated treatment at these depths in May and July, which was not the case (Table 13.19). In addition, *K. cristata* is known to have shallower rooting systems than *A. smithii* (Coupland and Johnson, 1965). If (b) were true, one would expect a similar response in September where a similar difference in *A. smithii* abundance between treatments was found. If (c) were true, one would have seen the reverse pattern in May, which was not the case.

Finding greater shoot biomass on the control plots with no treatment differences in root biomass indicates greater root/shoot ratios on treated plots and, therefore, a greater partitioning of photosynthate to roots of the treated plots. This would enhance the validity of the apparent stimulation. On the other hand, it may be indicative of an *a priori* difference between treatment plots caused by some other factor(s).

$^{35}SO_2$ Flux into Leaves

The most limiting step in the process of SO_2 diffusion into live leaves is stomatal resistance. Barley leaves with open stomates take up 6 times more SO_2 than leaves with closed stomates (Spedding, 1969), and greater SO_2 uptake by plants has been observed in lighted than in dark conditions (Garsed and Read, 1977). Capture of assimilated SO_2-sulfur depends also on leaf age. Thus, middle-aged leaves in tobacco captured more than either young expanding leaves or older leaves. However, this difference was not attributed to differences in stomatal opening between leaves of different ages (Craker and Starbuck, 1973). Guderian, (1970) found the same relationship but did attribute it to differences in stomatal opening between leaves of different ages.

Concentrations of SO_2 as low as 2.45 pphm may decrease stomatal resistance in *Vicia faba* (Biscoe *et al.*, 1973). Concentrations between 10 and 50 pphm also have been found to stimulate stomatal opening (Unsworth *et al.*, 1972). In moist air, stomates may be stimulated to open and in dry air to close in response to SO_2 (Majernik and Mansfield, 1972). Thus, humid conditions and SO_2 in the tent would have favored opening. I suggest that the greater SO_2 uptake by *A. smithii* and total live material on the fumigated treatment was caused by a stimulation of stomatal opening by SO_2. (SO_2 within the tents at labeling was almost entirely from captured ambient SO_2, SO_2 released from the label resulted in only nanograms-cm^{-3} concentrations.)

Factors Which Influence Sulfur Distribution

The distribution of sulfur in the plant under normal circumstances is governed by a combination of its mobility and the relative strength of sinks among plant parts. Free sulfur is mobile in the phloem (Bukovac and Wittwer, 1957) and the xylem but is captured quickly in metabolic processes and immobilized in areas with high growth rates (Biddulph *et al.*, 1958). Others have found that meristematic tissues of roots and leaves capture most foliar-applied SO_2 sulfur (Garsed and Read, 1974). Thus, the fate of sulfur is highly dependent on sink strengths created by growth demands. The distribution pattern is further influenced by the proximity of the source to the sink. Even with highly mobile phosphorus, translocation to roots from foliar application has been found to be related to the proximity of the fed leaves to the roots, with older more proximal leaves exporting more (Koontz and Biddulph, 1957). Another factor affecting the fate of SO_2 sulfur in perennial plants is the *relative* sink strengths of roots to shoots whereby roots may accumulate sulfur when sink strength in leaves is low because of senescence.

Distribution of Assimilated $^{35}SO_2$

On the control, a greater percentage of assimilated sulfur was sent to roots in September (14.8%) than in July (9.0%), which seems explainable in terms of aboveground growth demand, where the relative quantity of sulfur translocated to roots is expected to be dependent on the degree to which it is not needed by shoot growth. Relative shoot growth was significantly higher in July (Table 13.16) as was absolute growth (although not significantly). This indicates a more active shoot metabolism in July and thus an increased capacity to capture sulfur in growth; roots therefore received less.

The decline in translocation of SO_2 sulfur to 5-10 and 10-20 cm in September coupled with a simultaneous decline in root growth (Figure 13.33) supports the notion that lowered growth rates decrease sink strength. It does not, however, prove the theory, because the total quantity of recovered ^{14}C in the roots in July is not absolutely known to have been incorporated into structural components (as opposed to labile storage pools). Increase in translocation to the 0-5 cm layer along with the increased total sulfur at that depth indicates this layer became a more efficient sink as the season progressed. The cause for this sink strength appears not to lie in greater growth demands. Sulfur contents of these roots also seem not to be the cause as one would then have observed a treatment difference. Until captured by growth, sulfur is mobile in the plant (Biddulph *et al.*, 1958). Mobile ions are in a dynamic state with transport between tissues and between organs occurring continuously (Bowling, 1976). Lateral transport of ions between upward (xylem) and downward (phloem) transport pathways causes a circulation of ions in the plant (Weatherly, 1969). Thus, under conditions of lower growth demand at all rooting levels, the dynamic equilibrium between depths might have been governed more by the distribution of root biomass. Thus, the great concentration of roots in the 0-5 cm layer might help explain the increase in translocation to that depth in September.

Total Sulfur Concentrations

The relationship between root relative growth rates (growth in the loose sense) and total sulfur on the control treatment (Figure 13.33) indicates that during periods of high growth, total sulfur was lowest. Part of the explanation lies in a dilution of root sulfur by carbon growth, but the increasing sink strength of shoots between May and July may help explain the apparent draw-down in sulfur concentrations at 5-10 and 10-20 cm. On the fumigated treatment, there was a trend for accumulation of sulfur at 5-20 cm between May and July, possibly because shoots derived a major portion of their sulfur supply from the atmosphere. For example, cotton may derive 30% and ash seedlings 92% of their supply from SO_2 even with adequate sulfur levels in the rooting media (Jensen and Kozlowski, 1975). The lowered short demands may then have allowed 5-20 cm roots on the high treatments to accumulate sulfur.

Between July and September, all layers on control plots accumulated or maintained their sulfur levels despite a reduction in root growth. This was the period during which absolute aboveground growth rates declined (Table 13.16). As such, the sink strength for sulfur aboveground also declined, leaving the roots free to accumulate according to or in excess of their demands. The reverse trend was found below 5 cm on the fumigated plots over this time period, possibly because a feedback mechanism may operate to decrease root uptake when shoots have an adequate supply. Indeed, SO_2-fumigated plants do take less sulfur from the soil (Thomas *et al.*, 1943, Faller 1971).

Summary and Conclusions

The observed partitioning of growth among shoots, storage, organs, and different rooting depths supports the general hypothesis of a strong seasonal control. Greater proportions of photosynthate were sent to roots early in the season. Soil water showed more potential as the ultimate rather than the proximal cause of this relationship. Increasing soil temperatures compared to air temperatures, incident light, and possibly phenology might be more important as proximal cues. Maximal growth above and belowground generally occurred from early to mid season. Because approximately 48% of precipitation is normally received in April, May, and June (National Oceanic and Atmospheric Administration, 1976) these plants would profit most from a growth pattern that most fully exploits this resource. The C-3 photosynthetic pathway of the dominant species, which favors growth early in the season before the onset of higher late season temperatures, might be such an adaptation.

Distribution of root growth with depth seemed to be well correlated with soil water distribution. Early in the season, plants exhibited intense root activity nearest the surface, which would be advantageous for the immediate interception of rain events later in the season. The greater relative partitioning of growth to 5-20 cm late in the season seemed to be related to the more favorable conditions for root growth there as the top layers become more dry.

It appears that low concentrations of sulfur dioxide may have increased partitioning of photosynthate to 5-20 cm roots in mid season. The mechanism by which this effect would be brought about depends on a system of priorities among organs for capture of assimilate. If SO_2 sulfur has a fertilizing effect that stimulates photosynthesis more than shoot growth, root growth could be favored over shoot growth. The lack of a significant effect in the 0-5 cm layer suggested that other factors (such as soil drought) may have been restricting growth at this depth. Effects observed in this study probably represent the extreme case, as plots examined here were located on green patches at the time of the observed stimulation and, therefore, are representative only to the extent that the total system was covered with green biomass at that time. This stimulation is in contrast to findings for radish (Tingey *et al.*, 1971), possibly because of differing species susceptibilities to negative SO_2 effects, the difference between ideal growth chamber conditions and the stresses of the field, or because this system (or at least these study plots) indeed may have been sulfur limited. Only very intensive root biomass measurements made over an extended period of fumigation are likely to reveal the real extent and mechanism of the observed treatment effect.

Removal of SO_2 from the atmosphere by this system was governed to a large degree by the presence of aboveground biomass; however, litter and soil (fine litter) also played important roles. Deposition rates onto dead leaf surfaces were not markedly different than those onto live shoots, which suggests that estimates of removal based on stomatal resistance alone are not adequate. The removal process seemed to be controlled primarily by the magnitude of the absorbing surface area rather than by the gas exchange process of the plants. Increased removal rates by live material on fumigated plots over control plots suggested that stomatal opening may have been stimulated by SO_2. This increase was overshadowed, however, by the role played by passive deposition onto leaf surfaces, litter, and soil. The small quantities of total deposition found translocated to roots suggested that aboveground growth demands prevented large quantities from reaching roots.

Distribution of total sulfur in the plants was not unaffected by SO_2 fumigation. The suprising finding of lower sulfur contents in roots of fumigated plants suggested that root uptake was decreased as the source of sulfur shifted to the atmosphere. Fumigated shoots may have derived a significant portion of their sulfur from the atmosphere, thereby lessening the reliance on sulfur stored in deeper rooting layers. Thus, in the later part of the season, sulfur concentrations at 5-20 cm on the control treatment increased or remained constant but decreased or remained constant on the fumigated treatment. Roots in the 0-5 cm layer appeared to act as an efficient sink for sulfur translocated from shoots late in the season, yet there were lowered total sulfur concentrations in these roots on the fumigated plots. This result suggested that root uptake was governed by concentrations in shoots at least as much as by those in roots. It is possible that hormones controlling root uptake rates are manufactured in the shoots and translocated belowground when shoot demand is high.

Roots at 5-20 cm failed to accumulate sulfur on the fumigated treatment at the end of the growing season. This would not be detrimental to growth the following season provided the source of sulfur for aboveground growth remains in the atmosphere. Should the fumigation cease, however, there would be an increased reliance on rapid uptake from the soil. It is interesting to speculate what would happen to root sulfur over many years of fumigation. Perhaps it would decline to some minimal level as reliance on root uptake diminishes. Perhaps, as sulfur accumulates in the soil under fumigation, root uptake would again increase because of the response of Michaelis-Menten enzyme kinetics to increased substrate concentrations. The principal short-term effect appears to be a decrease in the strength of coupling of the plant to the soil with respect to sulfur. This also decreases the relationship of a plant to its neighbors with respect to this substrate. Perhaps real changes in community composition would occur in ecosystems where soil sulfur is deficient. Root uptake would no longer secure a competitive advantage to species short of sulfur. Composition would be expected to shift away from species with superior root uptake capabilities towards those with the capacity to derive their supply from the atmosphere. This assumes, of course, no side effects of SO_2 on metabolic machinery.

The overall response of this system to SO_2 fumigation at low levels was subtle indeed. Changes observed here could, if unchecked over many years, lead to fundamental changes in the structure and functioning of the ecosystem. Continued sulfur loading into this system eventually may facilitate H_2SO_4 acidification as the buffering capacity of the soil eventually is exceeded, which, in turn, may drive the system into a much different conformation. It is unknown how realistic, or how far in the future, such a response is.

A closing consideration is the extent to which this perturbation would be imposed on northern Great Plains ecosystems. It is likely that fossil fuel combustion in the region will continue to rise for some time in view of the vast reserves of coal located here and the increasing demand for electrical energy. According to Montana law, the maximum allowable annual arithmetic mean concentration is only 1/5 that used here (2 pphm), while the 24-hour maximum is identical to that used here (10 pphm). But these concentrations are more likely to be observed locally in the vicinity of the power facility, or at a distance as intermittent events. Because fumigation in this study was continuous, the observed responses probably would be confined to areas local rather than distant to SO_2 sources. More distant ecosystems would be less likely to be perturbed from their natural steady state because of the unreliability of SO_2 as a sulfur source or as an influence on primary production.

SIMULATION MODELS: SULFUR CYCLE AND ECOSYSTEM LEVEL MODEL*

During the past two years, an effort has been made to develop sulfur cycling and SO_2 deposition submodels and to incorporate them into an ecosystem level simulation model of the northern mixed-grass prairie. The ecosystem level model used was an improved version of the ELM model described by Innis (1978). The model was adapted to simulate the identical grassland type as that being studied by other Colstrip coal-firing power plant (CCFPP) investigators to determine the ecological effects of controlled field exposure to SO_2 (see Sections 9-20). The modeling work has been completed and will be reported in full in a dissertation by the first author later this year. The following is a summary report of results derived by exercising the model to test a number of hypotheses.

The ecosystem level model which includes abiotic, producer, and decomposer-nitrogen submodels was successfully constructed, and integrated with the sulfur cycling, and SO_2 deposition submodels. Comparisons of observed and simulated soil water dynamics, primary producer dynamics, shoot nitrogen, shoot sulfur, and litter biomass were generally favorably.

One exercise of the model involved a 2-year simulation run with ambient concentration of SO_2 held at 10 pphm throughout each growing season. SO_2 deposited sulfur had significant impacts on the cycling of sulfur. The total addition of sulfur amounted to $48.2 \text{ kg S} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$. The sum of all system flows of sulfur was increased by 3.2 and $5.8 \text{ g S} \cdot \text{m}^{-2}$ over each of the two growing seasons. When direct SO_2 fluxes are subtracted from these quantities, an indirect increase in total system sulfur flows becomes 1.1 and 3.1 $\text{g S} \cdot \text{m}^{-2}$. All sulfur state variables (standing crop of sulfur in various compartments) together were perturbed four to nine times over their normal deviations, indicating the sulfur cycling system to have deviated from steady-state behavior.

---

*Condensed from a dissertation by Michael B. Coughenour entitled "Grassland Sulfur Cycle and Ecosystem Responses to Low-Level SO_2."

Implementation of a restriction on plant growth at N/S ratios greater than 15 produced a significant model response to sulfur fertilization by SO_2. This response has been somewhat exaggerated over reality, but indicates the possibility is real. These results additionally showed that if aboveground growth is stimulated more than net photosynthesis, a decrease in belowground growth may be observed, which would explain the previously mentioned indications of a growth depression in rhizomes. Fertilization also brought about numerous secondary effects through the increase in net aboveground primary production.

Several other hypotheses regarding direct effects on primary producers were tested in the ecosystem context. An inhibition of photosynthesis decreased belowground growth much more than that above ground and this effect carried over on decomposer-related activity. A stimulation of photosynthesis increased production below ground and had no effect above ground. Increased senescence lowered peak standing crop, decreased total net photosynthesis, and depressed belowground growth.

DECOMPOSITION-LITTER BIOMASS DYNAMICS

Litter standing crop data for the two ZAPS sites are tabulated in Table 13.20. The general trend that is noted is an increase in the amounts of litter on all treatments over time. Litter will tend to increase on these sites to a new position of equilibrium as accumulation rates exceed decomposition rates now that cattle grazing has been prevented. Litter standing crop increased from 1976 to 1977 by 31% on ZAPS I and by 43% on ZAPS II, but on ZAPS I the increase from 1975 to 1976 was only 9%. An analysis of the yearly increases in litter on each treatment did not reveal any relationship to SO_2 treatment; however, at some time in the future we may be able to detect an effect by the SO_2 gas and sulfur content of the litter on decomposition rates.

TABLE 13.20. INTRASEASONAL DYNAMICS OF LITTER STANDING CROP*

Date	ZAPS I				ZAPS II				Average by date
	Control	Low	Medium	High	Control	Low	Medium	High	
20 April 1975	112 ± 6	112 ± 21	135 ± 16	39 ± 7					100
5 May 1975	102 ± 16	174 ± 21	168 ± 24	179 ± 23					156
15 June 1975	165 ± 8	137 ± 14	178 ± 17	124 ± 12					151
13 July 1975	123 ± 15	155 ± 17	155 ± 18	154 ± 16					147
13 August 1975	172 ± 17	192 ± 15	193 ± 17	189 ± 22					187
18 September 1975	156 ± 20	149 ± 19	193 ± 17	100 ± 33					175
Season average	138	153	170	148					
21 March 1976	126 ± 18	180 ± 12	167 ± 15	122 ± 10	146 ± 10	175 ± 16	155 ± 13	145 ± 13	152
17 May 1976	122 ± 11	176 ± 21	151 ± 12	152 ± 17	104 ± 8	149 ± 19	188 ± 17	152 ± 12	149
15 June 1976	130 ± 11	173 ± 13	154 ± 12	144 ± 17	133 ± 18	196 ± 23	172 ± 16	206 ± 27	164
10 July 1976	139 ± 10	170 ± 17	188 ± 10	174 ± 16	146 ± 8	242 ± 25	183 ± 21	223 ± 29	183
9 August 1976	212 ± 20	187 ± 17	188 ± 13	157 ± 10	196 ± 18	214 ± 20	201 ± 23	201 ± 24	195
17 September 1976	232 ± 17	181 ± 18	218 ± 26	141 ± 13	88 ± 11	107 ± 14	123 ± 14	142 ± 13	154
Season average	160	178	178	148	136	181	170	178	
12 July 1977	214 ± 13	241 ± 20	221 ± 16	191 ± 16	244 ± 15	231 ± 16	220 ± 16	251 ± 17	227

---

*$\bar{X}$ ± SE, oven-dry ash-free, g · m^{-2}

LITERATURE CITED

Almquist, E. 1974. An Analysis of Global Air Pollution. Ambio, 3(5):161-167.

Alway, F. S., A. W. Marsh, and W. J. Methley. 1937. Sufficiency of Atmospheric Sulfur for Maximum Crop Yields. Soil Sci. Soc. Am. Proc., 2:229-238.

Ares, J. 1976. Dynamics of the Root System of Blue Grama. J. Range Manage., 29:208-213.

Balasko, J. A., and D. Smith. 1973. Carbohydrates in Grasses: V. Incorporation of ^{14}C into Plant Parts and Nonstructural Carbohydrates of Timothy (*Phleum pratense* L.) at Three Developmental Stages. Crop Sci., 13:19-22.

Bailey, J. L., and R. D. Cole. 1959. Studies on the Reaction of Sulfite with Proteins. J. Biol. Chem., 234(7):1733-1739.

Barta, A. L. 1976. Transport and Distribution of $^{14}CO_2$ Assimilate in *Lolium perenne* in Response to Varying Nitrogen Supply to Halves of a Divided Root System. Plant Physiol., 38:48-52.

Barton, J. S., L. S. Bull, and R. W. Henten. 1971. Effects of Various Levels of Sulfur upon Cellulose Digestion in Purified Diets and Ligno-Cellulose Digestion in Corn Fodder Pellets *in vitro*. J. Anim. Sci., 33(3):682-685.

Bell, J. N. B., and W. S. Clough. 1973. Depression of Yield in Ryegrass Exposed to Sulfur Dioxide. Nature (Lond.), 241(5384):47-49.

Bennet, J. H., and A. C. Hill. 1973. Inhibition of Apparent Photosynthesis by Air Pollutants. J. Environ. Qual., 2(4):526-530.

Biddulph, O., S. Biddulph, R. Cory, and H. Koontz. 1958. Circulation Patterns for Phosphorus, Sulfur and Calcium in the Bean Plant. Plant Physiol., 33:293-300.

Biscoe, P. V., M. H. Unsworth, and H. R. Pickney. 1973. The Effects of Low Concentrations of Sulphur Dioxide on Stomatal Behavior in *Vicia faba*. New Phytol., 72:1299-1306.

Bleasdale, J. K. A. 1973. Effects of Coal-Smoke Pollution Gases on the Growth of Ryegrass (*Lolium perenne* L.). Environ. Pollut., 5(4):273-285.

Bokhari, U. G. 1977. Regrowth of Western Wheatgrass Utilizing ^{14}C-labeled Assimilates Stored in Belowground Parts. Plant Soil, 48:115-127.

Bowling, D. S. F. 1976. Uptake of Ions by Plant Roots. Chapman and Hall, London, England. 212 pp.

Bray, G. A. 1960. A Simple Efficient Liquid Scintillator for Counting Aqueous Solutions in a Liquid Scintillation Counter. Anal. Biochem., 1:279-285.

Brady, C. J. 1973. Changes Accompanying Growth and Senescence and Effects of Physiological Stress. In: Chemistry and Biochemistry of Herbage, G. W. Butler and R. W. Bailey, eds. Academic Press, Inc., New York. pp. 317-353.

Bromfield, A. R. 1972. Absorption of Atmospheric Sulphur by Mustard (*Sinapis alba*) Growth in a Glasshouse. J. Agric. Sci., 78:343-344.

Bukovac, M. S., and S. H. Wittwer. 1957. Absorption and Mobility of Foliar Applied Nutrients. Plant Physiol., 32:428-435.

Butters, B., and E. M. Chenery. 1959. A Rapid Method for the Determination of Total Sulphur in Soils and Plants. Analyst 84:239-245.

Caldwell, M. M., and L. B. Camp. 1974. Belowground Productivity of Two Cool Desert Communities. Oecologia, 17:123-130.

Cecil, R., and R. G. Wake. 1962. The Reaction of Inter- and Intra-chain Disulphide Bonds in Protein with Sulphite. Biochem. J., 82:401-406.

Clark, F. E. 1977. Internal Cycling of ^{15}Nitrogen in Shortgrass Prairie. Ecology, 58:1322-1333.

Coupland, R. T., and R. E. Johnson. 1965. Rooting Characteristics of Native Grassland Species in Saskatchewan. J. Ecol., 53:475-507.

Cowling, D. W., and D. R. Lockyer. 1976. Growth of Perennial Ryegrass (*Lolium perenne* L.) Exposed to a Low Concentration of Sulfur Dioxide. J. Exp. Bot., 27(98):411-417.

Cowling, D. W., L. H. S. Jones, and D. R. Lockyer. 1973. Increased Yield Through Correction of Sulphur Deficiency in Ryegrass Exposed to Sulphur Dioxide. Nature, 243:479-480.

Coyne, P. I., and C. W. Cook. 1970. Seasonal Carbohydrate Reserve Cycles in Eight Desert Range Species. J. Range Manage., 23:438-444.

Craker, L. E., and J. S. Starbuck. 1973. Leaf Age and Air Pollution Susceptibility: Uptake of Ozone and Sulfur Dioxide. Environ. Res., 6(1):91-94.

Crowley, D. H. 1975. Natural selection and the Michaelis constant. J. Theor. Biol., 50:461-475.

Dahlman, R. C., and C. L. Kucera. 1968. Tagging Native Grassland Vegetation with Carbon-14. Ecology, 49:1199-1203.

Dahlman, R. C., and C. L. Kucera. 1969. Carbon-14 Cycling in the Root and Soil Components of a Prairie Ecosystem. In: Symposium on Radioecology, D. J. Nelson and F. C. Evans, eds. U.S. Dep. Commerce, Springfield, Virginia. pp. 652-660.

Daines, R. H. 1968. Sulfur Dioxide and Plant Responses. J. Occup. Med., 10:516-524.

Davidson, R. L. 1969a. Effect of Root/leaf Temperature Differentials on Root/shoot Ratios in Some Pasture Grasses and Clover. Ann. Bot., 33:561-569.

Davidson, R. L. 1969b. Effects of Soil Nutrients and Moisture on Root/shoot Ratios in *Lolium perenne* L. and *Trifolium repens* L. Ann. Bot., 33:571-577.

Dodd, J. L., W. K. Lauenroth, R. K. Heitschmidt, and J. W. Leetham. 1978. First-year Effects of Controlled Sulfur Dioxide Fumigation on a Mixed-grass Prairie Ecosystem. In: The Bioenvironmental Impact of a Coal-fired Power Plant, E. M. Preston and R. A. Lewis, eds. 3rd Interim Report, EPA-600/3-78-021, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 345-375.

Evans, L. T., and I. F. Wardlaw. 1976. Aspects of the Comparative Physiology of Grain Yields in Cereals. Adv. Agron., 28:301-359.

Faller, N. 1970. Effects of Atmospheric SO_2 on Plants. Sulphur Inst. J., 6(4):5-7.

Faller, N. 1971. Plant Nutrient Sulphur-SO_2 *vs*. SO_4. Sulphur Inst. J., 7:5-6.

Fowler, D., and M. H. Unsworth. 1974. Dry Deposition of Sulphur Dioxide on Wheat. Nature, 249:389-390.

Garland, J. A., D. H. F. Atkins, C. J. Readings, and S. J. Coughey. 1974. Deposition of Gaseous Sulphur Dioxide to the Ground. Atmos. Environ., 8:75-79.

Garsed, S. G., and D. J. Read. 1974. The Uptake and Translocation of $^{35}SO_2$ in Soybean *Glycine max* var. *Biloxi*. New Phytol., 73:299-307.

Garsed, S. G., and D. J. Reed. 1977. Sulphur Dioxide Metabolism in Soybean *Glycine max* var. *Biloxi*: I. The Effects of Light and Dark on the Uptake and Translocation of $^{35}SO_2$. New Phytol., 78:111-119.

Guderian, R. 1970. Untersuchungen Ueber Quantitative Beziehungen Zwischen dem Schwefelgehalt von Pflanzen und dem Schwefeldioxidgehalt der Luft. Z. Pflanzenk. Pflanzenschutz, 77:289-308.

Guderian, R. 1977. Air Pollution. Springer-Verlag, New York. 127 pp.

Hammett, F. S. 1930. The Natural Chemical Regulation of Growth by Increase in Cell Number. Proc. Am. Philos. Soc., 69:217-223.

Heitschmidt, R. K. 1977. Chronic Effects of SO_2 on Western Wheatgrass in a Montana Grassland. Ph.D. Thesis, Colorado State University, Fort Collins, Colorado. 100 pp.

Hill, A. C. 1971. Vegetation: A Sink for Atmospheric Pollutants. J. Air Pollut. Control Assoc., 21:341-346.

Hubbert, F., Jr., E. Cheng, and W. Burroughs. 1955. Mineral Requirements of Rumen Microorganisms for Cellulose Digestion in vitro. J. Anim. Sci., 17(3):559-568.

Hunt, C. H., O. G. Bentley, T. V. Hershberger, and J. H. Cline. 1954. The Effect of Carbohydrates and Sulfur on B-vitamin Synthesis, Cellulose Digestion, and Urea Utilization by Rumen Microorganisms in Vitro. J. Anim. Sci., 13(3):570-580.

Innis, G. S. 1978. Grassland Simulation Model. Ecol. Studies No. 26. Springer-Verlag, New York. 298 pp.

Jeffay, H., and J. Alvarez. 1961a. Liquid Scintillation Counting of Carbon-14. Anal. Chem., 33:612-615.

Jeffay, H., and J. Alvarez. 1961b. Measurement of C^{14} and S^{35} in a Single Sample. J. Anal. Biochem., 2:506-508.

Jensen, K. F., and T. T. Kozlowski. 1975. Absorption and Translocation of Sulfur Dioxide by Seedlings of Four Forest Tree Species. J. Environ. Qual., 4:379-382.

Jewiss, O. R., and J. Woledge. 1967. The Effect of Age on the Rate of Apparent Photosynthesis in Leaves of Tall Fescue (*Festuca arundinacea* Schreb.). Ann. Bot. (N.S.), 31:661-671.

Katz, M. 1949. Sulfur Dioxide in the Atmosphere and its Relation to Plant Life. Ind. Eng. Chem., 41(11):2450-2465.

Kellogg, W. W., R. D. Codle, E. R. Allen, A. L. Lazrus, and E. A. Martell. 1972. The Sulfur Cycle. Science, 175(11):587-596.

Koontz, H., and O. Biddulph. 1957. Factors Affecting Absorption and Translocation of Foliar Applied Phosphorus. Plant Physiol., 32:463-470.

Krober, O., and R. W. Howell. 1958. Determination of Sulfur in Plant Materials. Agric. Food Chem., 6:591-592.

Kulman, H. M. 1971. Effects of Insect Defoliation on Growth and Mortality of Trees. Annu. Rev. Entomol., 16:289-324.

Lauenroth, W. K., J. L. Dodd, R. K. Heitschmidt, and R. G. Woodmansee. 1975. Biomass Dynamics and Primary Production in Mixed Prairie Grasslands in Southeastern Montana: Baseline Data for Air Pollution Studies. In: Proceedings of the Fort Union Coal Field Symposium, W. Clark, ed. Eastern Montana Coll., Billings. pp. 559-578.

Laycock, W. A. 1967. How Heavy Grazing and Protection Affect Sagebrush-grass Ranges. J. Range Manage., 20(4):206-213.

Lee, J. J., R. A. Lewis, and D. E. Body. 1976. The Field Experimental Component: Evaluation of the Zonal Air Pollution System. EPA Ecological Research Series EPA-600/3-76-013, Environmental Res. Lab., Office of Research and Development. U.S. Environmental Protection Agency, Duluth, Minnesota. pp. 188-202.

Loosli, J. K., H. H. Williams, W. E. Williams, W. E. Thomas, F. H. Ferris, and L. A. Maynard. 1949. Synthesis of Amino Acids in the Rumen. Science, 110:144-145.

Lovelock, J. F., and L. Margulis. 1974. Atmospheric Homeostasis by and for the Biosphere: The Gaia Hypothesis. Tellus, 26:1-9.

Lupton, F. G. H. 1968. The Analysis of Grain Yield of Wheat in Terms of Photosynthetic Ability and Efficiency of Translocation. Ann. Appl. Biol., 61:108-119.

Lush, W. M., and L. T. Evans. 1974. Translocation of Photosynthetic Assimilate from Grass Leaves as Influenced by Environment and Species. Aust. J. Plant Physiol., 1:417-431.

Majernik, O., and T. A. Mansfield. 1972. Stomatal Responses to Raised Atmospheric CO_2 Concentrations during Exposure of Plants to SO_2 Pollution. Environ. Pollut., 3:1-7.

Malhorta, S. S., and D. Hocking. 1976. Biochemical and Cytological Effects of Sulfur Dioxide on Plant Metabolism. New Phytol., 76:227-237.

Martin, J. E., L. R. Arrington, J. E. Moore, C. B. Ammerman, G. K. Davis, and R. L. Shirley. 1964. Effect of Magnesium and Sulfur upon Cellulose Digestion of Purified Rations by Cattle and Sheep. J. Nutr., 83:60-64.

McDougall, E. I. 1948. Studies on Ruminant Saliva. 1. The Composition and Output of Sheep's Saliva. Biochem. J., 43(1):99-109.

Mitchell, K. J. 1954. Influence of Light and Temperature on the Growth of Ryegrass (*Lolium* sp.). III. Pattern and Rate of Tissue Formation. Physiol. Plant 7:51-65.

Moss, D. N., and D. E. Peaslee. 1965. Photosynthesis of Maize Leaves as Affected by Age and Nutrient Status. Crop Sci., 5:280-281.

National Oceanic and Atmospheric Administration. 1976. Climatological Data, Montana Annual Summary, 1976. U.S. Dep. Commerce, Washington, D. C. Vol. 79, No. 13. 19 pp.

Olsen, R. 1957. Absorption of Sulfur Dioxide from Atmosphere by Cotton Plants. Soil Sci., 84:107-111.

Owers, M. J., and A. W. Powell. 1974. Deposition Velocity of Sulphur Dioxide on Land and Water Surfaces Using a ^{35}S Tracer Method. Atmos. Environ., 8:63-67.

Pearson, H. A. 1970. Digestibility Trials: *In vitro* Techniques. Range and Wildlife Habitat Evaluation--A Research Symposium. USDA Misc. Pub. No. 1147. pp. 85-92.

Radford, P. J. 1967. Growth Analysis Formulae - Their Use and Abuse. Crop Sci., 7(3):171-175.

Risser, P. G., and F. L. Johnson. 1973. Carbon Dioxide Exchange Characteristics of Some Prairie Grass Seedlings. Southwest. Nat., 18:89-91.

Ryle, G. J. A. 1970a. Distribution Patterns of Assimilated ^{14}C in Vegetative and Reproductive Shoots of *Lolium perenne* and *L. temulentum*. Ann. Appl. Biol., 66:155-167.

Ryle, G. J. A. 1970b. Partition of Assimilates in an Annual and a Perennial Grass. J. Appl. Ecol., 7:217-227.

Ryle, G. J. A., and C. E. Powell. 1976. Effect of Rate of Photosynthesis on the Pattern of Assimilate Distribution in the Graminaceous Plant. J. Exp. Bot., 27:189-199.

Scholander, P. F., H. T. Hammel, E. D. Broadstreet, and E. A. Hemmingen. 1965. Sap Pressure in Vascular Plants. Science, 148:339-346.

Schwartz, C. C., W. K. Lauenroth, R. K. Heitschmidt, and J. L. Dodd. 1978. Effects of Controlled Levels of Sulphur Dioxide on the Nutrient Quality of Western Wheatgrass. J. Appl. Ecol., 15(3):869-874.

Seim, E. 1970. Sulfur Dioxide Absorption by Soil. Ph.D. Thesis, University of Minnesota, Minneapolis, Minnesota. 138 pp.

Shimshi, D. 1969. A Rapid Field Method for Measuring Photosynthesis with Labelled Carbon Dioxide. J. Exp. Bot., 20:381-401.

Siji, J. W., and C. A. Swanson. 1974. Short-term Kinetic Studies on the Inhibition of Photosynthesis by Sulfur Dioxide. J. Environ. Qual., 3(2):103-107.

Singh, J. S., and D. C. Coleman. 1973. A Technique for Evaluating Functional Root Biomass in Grassland Ecosystems. Can. J. Bot., 51:1867-1870.

Singh, J. S., and D. C. Coleman. 1974. Distribution of Photoassimilated ^{14}Carbon in the Root System of a Shortgrass Prairie. J. Ecol., 62:359-365.

Singh, J. S., and D. C. Coleman. 1977. Evaluation of Functional Root Biomass and Translocation of Photoassimilated ^{14}C in a Shortgrass Prairie Ecosystem. In: The Belowground Ecosystem--A Synthesis of Plant-Associated Processes, J. K. Marshall, ed. Range Sci. Dep. Sci. Ser. No. 26, Colorado State University, Fort Collins, Colorado. pp. 123-131.

Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State University Press, Ames, Iowa. 593 pp.

Sosebee, R. E., and H. H. Wiebe. 1971. Effect of Water Stress and Clipping on Photosynthate Translocation in Two Grasses. Agron. J., 63:14-17.

Spedding, D. J. 1969. Uptake of Sulphur Dioxide by Barley Leaves at Low Sulphur Dioxide Concentrations. Nature, 224:1229-1231.

Starks, P. B., W. H. Hale, U. S. Garrigus, R. M. Forbes, and M. F. James. 1954. Response of Lambs Fed Varied Levels of Elemental Sulfur, Sulfate Sulfur, and Methionine. J. Anim. Sci., 13(1):249-257.

Stewart, B. A., and C. J. Whitfield. 1965. Effects of Crop Residue, Soil Temperature and Sulfur on the Growth of Winter Wheat. Soil Sci. Soc. Am. Proc., 29:752-755.

Swain, R. E., and A. B. Johnson. 1936. Effect of Sulfur Dioxide on Wheat Development. Ind. Eng. Chem., 28:42-47.

Thomas, M. D. 1956. The Invisible Injury Theory of Plant Damage. J. Air Pollut. Control Assoc., 5:205-208.

Thomas, M. D., and R. H. Hendricks. 1956. Effects of Air Pollution on Plants. In: Air Pollution Handbook, P. L. Magill, F. Holden, and C. Ackley, eds. McGraw-Hill Book Co., Inc., New York. pp. 9.1-9.44.

Thomas, M. D., R. H. Hendricks, T. R. Collier, and G. R. Hill. 1943. The Utilization of Sulfur Dioxide for the Sulfur Nutrition of Alfalfa. Plant Physiol., 18:345-371.

Thomas, M. D., R. H. Hendricks, and G. R. Hill. 1950. Sulfur Metabolism of Plants. Ind. Eng. Chem., 42(11):2231-2235.

Thomas, W. E., J. K. Loosli, H. H. Williams, and L. A. Maynard. 1951. The Utilization of Inorganic Sulfates and Urea Nitrogen by Lambs. J. Nutr., 43(4):515-523.

Thorne, G. N. 1963. Varietal Differences in Photosynthesis of Ears and Leaves of Barley. Ann. Bot., 27:155-174.

Tieszen, L. L., D. A. Johnson, and M. M. Caldwell. 1974. A Portable System for the Measurement of Photosynthesis Using 14-Carbon Dioxide. Photosynthetica, 8:151-160.

Tilley, J. M. A., and A. Terry. 1963. A Two-stage Technique for the *in vitro* Digestion of Forage Crops. J. Br. Grassl. Soc., 18:104-111.

Tingey, D. T. 1974. Ozone Induced Alterations in the Metabolite Pools and Enzyme Activities of Plants. In: Air Pollution Effects on Plant Growth, M. Dogger, ed. Am. Chem. Soc., Washington, D. C. pp. 40-57.

Tingey, D. T., W. W. Heck, and R. A. Reinert. 1971. Effect of Low Concentrations of Ozone and Sulfur Dioxide on Foliage, Growth and Yield of Radish. J. Am. Soc. Hortic. Sci., 96:369-371.

Tingey, D. T., R. W. Field, and L. Bard. 1976. Physiological Responses of Vegetation to Coal-fired Power Plant Emissions. In: The Bioenvironmental Impact of a Coal-fired Power Plant: Second Interim Rep. Colstrip, Montana. EPA 600/3-76-013, Environmental Protection Agency Office of Research and Development and Corvallis Environ. Res. Lab., Corvallis, Oregon. pp. 100-111.

Tomanek, G. W., and F. W. Albertson. 1953. Some Effects of Grazing on Mixed Prairie Near Hays, Kansas. J. Range Manage., 6(5):299-306.

Trenkle, A., E. Cheng, and W. Burrough. 1958. Availability of Different Sulfur Sources for Rumen Micro-organisms in *In vitro* Cellulose Digestion. J. Anim. Sci., 17: 1191.

Trlica, M. J., and C. W. Cook. 1972. Carbohydrate Reserves of Crested Wheatgrass and Russian Wildrye as Influenced by Development and Defoliation. J. Range Manage., 25:430-435.

Ulrich, A., M. A. Tabatabai, K. Ohki, and C. M. Johnson. 1967. Sulfur Content of Alfalfa in Relation to Growth in Filtered and Unfiltered Air. Plant Soil, 26:235-252.

Unsworth, M. H., P. V. Biscoe, and H. R. Pinckney. 1972. Stomatal Responses to Sulphur Dioxide. Nature (Lond.), 239:458-459.

Van Soest, P. J., and R. H. Wine. 1967. Use of Detergents in the Analysis of Fibrous Feeds. IV. Determination of Plant Cell-wall Constituents. J. Assoc. Off. Anal. Chem., 50(1):50-55.

Wardlaw, I. F. 1968. The Control and Pattern of Movement of Carbohydrate in Plants. Bot. Rev., 34:79-105.

Wardlaw, I. F. 1969. The Effect of Water Stress on Translocation in Relation to Photosynthesis and Growth. Aust. J. Biol. Sci., 22:1-16.

Wardlaw, I. F. 1976. Assimilate Movement in *Lolium* and Sorghum Leaves. I. Irradiance Effects on Photosynthesis, Export and the Distribution of Assimilates. Aust. J. Plant Physiol., 3:377-387.

Warembourg, F. R., and E. A. Paul. 1973. The Use of $C^{14}O_2$ Canopy Techniques for Measuring Carbon Transfer Through the Plant-Soil System. Plant Soil, 38:331-345.

Weatherly, P. E. 1969. Ion Movement Within the Plant and Its Integration with Other Physiological Processes. In: Ecological Aspects of the Mineral Nutrition of Plants, I. H. Rorison, ed. Blackwell Scientific Publications, Oxford, England. pp. 323-340.

Weinmann, H. 1961. Total Available Carbohydrates in Grasses and Legumes. Herbage Abstr., 31:255-261.

Whelpdale, D. M., and R. W. Shaw. 1974. Sulphur Dioxide Removal by Turbulent Transfer over Grass, Snow, and Water Surfaces. Tellus, 26:196-204.

Williams, G. J., III. 1974. Photosynthetic adaptation to temperature in C_3 and C_4 grasses--A possible ecological role in the shortgrass prairie. Plant Physiol., 54:709-711.

Williams, R. F. 1955. Redistribution of Mineral Elements During Development. Annu. Rev. Plant Physiol., 6:25-42.

Williams, R. F. 1965. Assimilation and Translocation in Perennial Grasses. Ann. Bot., 28:419-425.

Winer, B. J. 1971. Statistical Principles in Experimental Design, 2nd ed. McGraw-Hill Book Co., Inc., New York. 907 pp.

Zelitch, I. 1960. Synthesis of Glycolic Acid by Spinach Chloroplasts. Biochem. J., 77:11.

Ziegler, I. 1975. The Effects of SO_2 Pollution on Plant Metabolism. Residue Rev., 56:79-105.

TABLE 13.1. PHENOLOGY AT ZAPS I, 1976 AND 1977 (A = CONTROL, B = LOW, C = MEDIUM, D = HIGH)

Date	*Achillea millifolium*				*Agropyron smithii*				*Antennaria rosea*				*Artemisia frigida*				*Aristida longiseta*				*Bouteloua gracilis*				*Bromus japonicus*			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
18-3-76	1	1	1	1	1	1	1	1	12	12	12	12				1	12	12	12	12					2	2	2	2
29-3-76	1	1	1	1	2	2	2	2	2	2	2	2		1	1	1	12	12	12	12	12	12	12	12	2	2	2	2
12-4-76	2	2	2	2	2	2	2	2					2	3		2												
19-4-76	2	2	2	2	3	3	3	3																				
30-4-76	2	2	2	2	3	3	3	3																				
10-5-76	3	3	3	3	3	3	3	3																				
15-5-76	5	5	5	5	3	3	3	3	9	9	9	9	2	2	2	2	1	1	1	1		2	2		3			
26-5-76	6	6	6	6	3	3	3	3	9	9	9	9	2	2	2	2	1	1	1	1					4	4	4	4
2-6-76	7	8	7	7	3	3	3	3	9	10	10	10	3	3	3	3	1	1	1	1		3			5	5	5	5
10-6-76	7	8	8	7	3	3	3	3	11	11	11	11	3	3	3	3	3	3	3	2		3		3	6	6	6	6
16-6-76	8	8	8	8	4	4	4	4	11	11	11	11	4	3	3	3	5	5	5	5					6	6	6	6
24-6-76	8	8	8	8	4	5	4	4	11	11	11	11	4	4	4	4	6	6	6	6	4	4	4	4	6	6	6	6
1-7-76	8	8	8	8	5	5	5	5	12	11	11	12	4	4	4	4	6	6	6	6	4	4	4	4	8	8	8	8
8-7-76	8	8	8	8	6	6	6	6		11	11		4	4	4	4	6	6	6	6	6	6	7	7	9	9	9	9
15-7-76	8	8	8	8	7	7	7	7		12	12		4	4	4	4	7	7	7	7	7	7	7	7	9	9	9	9
22-7-76	8	8	11	8	8	8	8	8						4	4	4	9	9	9	9	8	8	8	8	9	9	9	9
29-7-76	11	11	11	11	9	9	9	9					5	5	5	5	10	10	10	10	9	9	9	9	11	11	11	11
5-8-76	11	11	12	11	9	9	9	9					6	6	6	6	11	11	11	11	10	10	10	10	11	11	11	11
12-8-76	12	12		12	10	10	9	10					6	6	6	7	11	11	11	11	10	10	10	10	11	11	11	11
19-8-76					10	10	10	10					7	7	7	7	11	11	11	11	11	11	11	11	12	12	12	12
26-8-76					11	11	11	11					7	7	7	7	12	12	11	12	12	12	11	12				
3-9-76					11	11	11	12					7	7	7	7			12				12					
9-9-76					11	11	12						8	8	7	8												
11-4-77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	--	--	--	0	--	--	--	1	1	1	1
19-4-77	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	--	--	--	--	--	--	--	--	1	1	1	1
25-4-77	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0	1	1	1	0	0	0	--	2	2	2	2
2-5-77	3	3	3	3	3	3	3	3	2	2	2	2	3	3	3	3	1	1	1	1	1	1	1	1	3	3	3	3
9-5-77	5	5	5	5	3	3	3	3	5	5	5	5	3	3	3	3	2	2	2	2	2	2	2	2	4	4	4	4
16-5-77	5	5	5	5	3	3	3	3	5	5	5	5	3	3	3	3	3	3	3	3	3	3	3	3	4	4	4	4
24-5-77	6	6	6	6	3	3	3	3	9	9	9	9	3	3	3	3	3	3	3	3	3	3	3	3	4	4	4	4
7-6-77	8	8	8	8	4	4	4	4	10	10	10	10	3	3	3	3	--	4	4	4	4	4	4	4	7	7	7	7
15-6-77	8	8	8	8	4	4	4	4	10	10	10	10	4	4	4	4	--	5	5	5	4	4	4	4	8	8	8	8
24-6-77	9	9	9	9	4	4	4	4	11	11	11	11	4	4	4	4	7	7	7	7	4	4	4	4	9	9	9	9
30-6-77	9	9	9	9	5	5	5	5	12	12	12	12	4	4	4	4	8	8	8	8	6	6	6	6	9	9	9	9
7-7-77	9	9	9	10	6	6	7	--	--				4	4	4	--	9	9	9	9	7	7	7	--	10	10	10	--
22-7-77	11	11	11	11	8	8	8	8					4	4	4	4	9	9	9	9	9	9	9	9	12	12	12	12
6-8-77	12	12	12	12	9	9	9	9					5	5	5	5	10	10	10	10	10	10	10	10				
12-8-77					11	11	10	10					5	5	5	5	11	11	11	11	11	11	11	11				
18-8-77					11	11	11	11					5	5	5	5	11	11	11	11	11	11	11	11				
25-8-77					11	11	11	11					7	7	7	6	11	11	11	11	11	11	11	11				
1-9-77					11	11	11	11					7	7	7	8	11	11	11	11	11	11	11	11				
8-9-77					11	11	11	11					7	7	7	8	11	11	11	11	11	11	11	11				
15-9-77					11	11	11	11					7	8	7	9	11	11	11	11	11	11	11	11				
22-9-77					11	11	11	11					8	9	8	9	11	11	11	11	11	11	11	11				

TABLE 13.1. (CONTINUED)

Date	*Cerastium arvense*				*Koeleria cristata*				*Lepidium deneiflorum*				*Phlox hoodi*				*Plantago patagonia*				*Poa pratensis*				*Poa secunda*			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
18-3-76					2	2	2	2																	3	3	3	3
29-3-76					3	3	3	3																	3	3	3	3
12-4-76					3	3	3	3																	3	3	3	3
19-4-76					3	3	3																		3	3		
30-4-76																												
10-5-76							4	4																	5	5	5	5
15-5-76	8	8			5	5	5	5																	6	6	6	6
26-5-76	8	8	8	8	6	6	6	6	11	11	11	11													6	6	6	6
2-6-76	8	8	8	8	6	6	6	6	11	11	11	11													7	7	7	7
10-6-76	11	11	11	11	7	7	7	7	12	12	11	12					4	4	4	4					8	8	8	8
16-6-76	11	11	11	11	7	7	7	7			12						5	5	5						8	8	8	8
24-6-76	11	11	11	11	8	8	8	8									7	7	7	7					8	9	9	9
1-7-76	12	12	12	12	9	9	9	9									8	8	8	8					11	11	11	11
8-7-76					10	10	10	10									8	8	8	8					11	11	11	11
15-7-76					11	10	10	11									8	8	8	8					12	12	12	12
22-7-76					11	11	11	11									10	10	10	10								
29-7-76					11	11	11	11									10	10	10	11								
5-8-76					12	12	12	12									11	11	11	11								
12-8-76																	11	11	11	11								
19-8-76																	12	12	12	12								
26-8-76																												
3-9-76																												
9-9-76																												
11-4-77					1	1	1	1																	2	2	2	2
19-4-77					1	1	1	1																	2	2	2	2
25-4-77					2	2	2	2																	3	3	3	3
2-5-77					3	3	3	3					8	8	8	8									3	3	3	3
9-5-77					4	4	4	4					9	9	9	9									6	6	6	6
16-5-77					6	6	6	6					10	10	10	10									--	--	--	--
24-5-77					7	7	7	7					10	10	10	10					8	8	8	8	8	8	8	8
7-6-77					8	8	8	8					11	11	11	11					8	8	8	8	8	8	8	8
15-6-77					8	8	8	8					12	12	12	12					--	--	--	--	9	9	9	9
24-6-77					9	9	9	9					12	12	12	12					--	--	--	--	10	10	10	10
30-6-77					10	10	10	10													11	11	11	11	11	11	11	11
7-7-77					11	11	11	--													12	12	12	--	12	12	12	12
22-7-77					12	12	12	12																				
6-8-77																												
12-8-77																												
18-8-77																												
25-8-77																												
1-9-77																												
8-9-77																												
15-9-77																												
22-9-77																												

TABLE 13.1. (CONTINUED)

Date	*Psoralea argophylla* A	B	C	D	*Sphaeralcea coccinae* A	B	C	D	*Stipa comata* A	B	C	D	*Stipa viridula* A	B	C	D	*Taraxacum officinale* A	B	C	D	*Tragopogon dubius* A	B	C	D	*Vicia americana* A	B	C	D
18-3-76					12	12	12		1	1	1	1					1	1	1	1	2		2	2				
29-3-76	12				12	12	1		1	1	1	1	1				2	2	2	2	2	2	2	2				
12-4-76																	5	5	5	5	3	3	3	3				
19-4-76																	5	6	6	6	3	3	3					
30-4-76																	7	7	7	7								
10-5-76																	8	8	9	8								
15-5-76					3	2	2		3	3	3	3	2		2		10	10	10	10	5		5	5		8		
26-5-76		3			3	3	3	3	3	3	3	3					10	10	11	11	5	5	5	5	7	7	8	8
2-6-76					4	4	4	4	3	3	3	3	4		4		11	11	11	11	6	6	6	6				8
10-6-76	3	3	4	4	5	5	5	5	7	7	8	7	6	6	6	6	11	11	11	11	7	7	7	7	11			
16-6-76	4	4	5	5	5	5	5	5	8	8	8	7	6	6	6	6	11	11	11	11	8	8	8	8				
24-6-76	6	6	5	6	9	9	9	9	9	9	9	9	7			7	11	11	11	11	8	8	8	9	9			11
1-7-76	9	10	10	9	9	9	9	9	10	10	10	10	9	9	9	9	11	11	11	11	10	10	10	10	11	11	11	11
8-7-76	10	10	10	10	9	9	9	9	10	10	10	10	11	10	10	10	11	11	11	11	10	10	10	10	11	11	11	11
15-7-76	10	10	10	10	10	10	9	9	11	11	11	11	11	10	10	10	11	11	11	11	10	10	10	10	11	11	11	11
22-7-76	10	10	10	10	10	10	10	10	11	11	11	11	11	11	11	11	11	11	11	11	10	10	10	10	11	11	11	11
29-7-76	10	10	10	10	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	10	11	11	11	11	11	11	11
5-8-76	10	10	10	10	11	11	11	11	12	12	12	12	11	11	11	11	11	11	11	11	10	11	11	11	12	12	12	12
12-8-76	11	11	11	11	11	11	11	11					12	12	12	12	12	12	12	12	11	11	11	11				
19-8-76	11	11	11	11	12	12	12	12													12	12	12	12				
26-8-76																												
3-9-76																												
9-9-76	12	12	12	12					1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
									1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	1	1	1	1
11-4-77					4	4	4	--	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
19-4-77					--	--	--	--	--	--	--	--	--	--	--	--	8	8	8	8	3	3	3	3	--	--	--	--
25-4-77			1		--	--	--	--	4	4	4	4	3	3	3	3	9	9	9	9	3	3	3	3	8	8	8	8
2-5-77	1	1	1	1	--	--	--	--	4	4	4	4	--	--	--	--	10	10	10	10	4	4	4	4	8	8	8	8
9-5-77	--	--	--	--	4	4	4	4	4	4	4	4	--	--	--	--	11	11	11	11	4	4	4	4	8	8	8	8
16-5-77	--	--	4	--	5	5	5	5	6	6	6	6	6	6	6	6	11	11	11	11	9	9	9	9	8	8	8	8
24-5-77	4	4	4	4	4	4	4	4	7	7	7	7	6	6	6	6	12	12	12	12	9	9	9	9	9	9	9	9
7-6-77	4	4	4	4	6	6	6	6	9	9	9	9	9	9	9	9	12	12	12	12	10	10	10	10	10	10	10	10
15-6-77	5	5	5	5	8	8	8	8	10	10	9	10	10	10	10	10					10	10	10	10	--	10	--	--
24-6-77	8	8	8	8	9	9	9	9	10	10	10	--	10	10	10	--					11	11	11	--	10	10	10	--
30-6-77	8	8	8	8	9	9	10	9	11	11	11	11	11	11	11	11					12	12	12	12	12	12	12	12
7-7-77	9	9	9	--	10	10	10	-	12	12	12	12	12	12	12	12												
22-7-77	9	9	9	9	11	11	11	11																				
6-8-77	12	12	12	12	12	12	12	12																				
12-8-77																												
18-8-77																												
25-8-77																												
1-9-77																												
8-9-77																												
15-9-77																												
22-9-77																												

TABLE 13.2. PHENOLOGY AT ZAPS II, 1976 AND 1977 (A = CONTROL, B = LOW, C = MEDIUM, D = HIGH)

	Achillea millifolium				*Agropyron smithii*				*Antennaria rosea*				*Artemisia frigida*				*Aristida longiseta*				*Bouteloua gracilis*				*Bromus japonicus*			
Date	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
18-3-76	1	1	1	1	1	1	1	1					1	1	1	1									2	2	2	2
29-3-76	1	1	1	1	2	2	2	2					1	1	1	1					1	1	1	1	2	2	2	2
12-4-76	2	2	2	2	3	3	3	3					3															
19-4-76	2	2	2	2																								
2-5-76	2	2	2	2	3	3	3	3					3															
10-5-76	3	3	3	3	3	3	3	3					3															
15-5-76	5	5	5	5	3	3	3	3			3		3	3	3	3					2		2	2	3	3		3
26-5-76	6	6	6	6	3	3	3	3	8	8	8	8	3	3	3	3									5	5	5	5
2-6-76	7	7	7	7	3	3	3	3	10	10	10	10	3	3	3	3	1	1	1	1				3	5	5	5	5
10-6-76	7	7	7	7	3	3	4	3	11	11	11	11	3	3	3	3	2	2	2	2					6	6	6	6
16-6-76	8	8	8	8	4	3	4	4	11	11	11	11	4	3	3	3	5	5	5	5	3		3	3	6	6	6	6
24-6-76	8	8	8	8	5	4	4	4	11	11	11	11	4	4	4	4	6	6	6	6	4	4	4	4	6	6	6	6
1-7-76	8	8	8	8	5	5	5	5	11	11	11	11	4	4	4	4	6	6	6	6	4	4	4	5	8	8	8	8
8-7-76	8	8	8	8	6	6	6	6	11	11	11	11	4	4	4	4	6	6	6	6	7	7	7	7	9	9	8	9
15-7-76	8	8	8	8	6	7		8	12	12	12	12	4	4	4	4	7	7	7	7	7	7	7	7	9	9	9	9
22-7-76	8	8	8	8	8	8	8	8					4	4	4	4	9	9	9	9	8	8	8	8	10	10	10	10
29-7-76	11	11	11	11	9	9	9	9					5	5	5	5	10	10	10	10	9	9	9	9	11	11	11	11
5-8-76	11	11	11	11	9	9	9	9					6	6	6	6	11	11	11	11	10	10	10	10	11	11	11	11
12-8-76	12	12	12	12	10	10	10	10					6	6	6	6	11	10	11	11	10	10	10	10	11	11	11	11
19-8-76					10	11	11	11					7	7	7	7	11	11	11	11	11	11	11	11	12	12	12	12
26-8-76					11	11	11	11					7	7	7	7		11	11		11	12	12	11				
3-9-76					11	12	12	12					7	7	8	7					12			12				
9-9-76					11								8	8	8	7		12	12									
11-4-77	1	1	1	1	1	1	1	1					1	1	1	1					-	-	-		1	1	1	1
19-4-77	1	1	1	1	1	1	1	1					2	2	2	2					-	-	-		1	1	1	1
25-4-77	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2					-	-	-		2	2	2	2
2-5-77	4	4	4	4	3	3	3	3					3	3	3	3					1	1	1	1	3	3	3	3
9-5-77	5	5	5	5	3	3	3	3					3	3	3	3					2	2	2	2	4	4	4	4
16-5-77	5	5	5	5	3	3	3	3					3	3	3	3					3	3	3	3	4	4	4	4
24-5-77	6	6	6	6	3	3	3	3					3	3	3	3					3	3	3	3	6	6	6	6
7-6-77	8	8	8	8	4	4	4	4					3	3	3	3					4	4	4	4	7	7	7	7
15-6-77	8	8	8	8	4	4	4	4					4	4	4	4					4	4	4	4	8	8	8	8
24-6-77	9	9	9	9	4	4	4	4					4	4	4	4					4	4	4	4	9	9	9	9
30-6-77	9	9	9	9	5	5	5	5					5	5	5	5					6	6	6	6	11	11	11	11
7-7-77	10	10	10	10	7	7	6	7					5	5	5	5					7	7	7	7	12	12	12	12
22-7-77	11	11	11	11	8	8	8	8					5	5	5	5					9	9	9	9				
6-8-77	12	12	12	12	10	9	9	9					5	5	5	5					10	10	10	10				
12-8-77					11	10	10	10					5	6	6	6					11	11	11	11				
18-8-77					11	11	11	11					6	6	6	8					11	11	11	12				
25-8-77					11	11	11	11					6	7	6	8					11	11	11	11				
1-9-77					11	11	11	11					6	6	6	9					11	11	11	11				
8-9-77					11	11	11	11					8	7	6	10					11	11	11	11				
15-9-77					11	11	11	11					7	8	6	10					11	11	11	11				
22-9-77					11	11	11	11					8	9	9	10					11	11	11	11				

TABLE 13.2. (CONTINUED)

Date	*Cerastium arvense*				*Koeleria cristata*				*Lepidium densiflorum*				*Lomatium orientale*				*Phlox hoodi*				*Plantago patagonia*				*Poa pratensis*			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
18-3-76					2	2	2	2																				
29-3-76					2	2	2	2																				
12-4-76					3			3																				
19-4-76																												
2-5-76																												
10-5-76					4	4	5	3																				
15-5-76					5	5	5	5	9		9	9																
26-5-76	7	8	8	8	6	6	6	6	9	9	9	9																
2-6-76	8	8	8	8	6	6	6	6	10	10	10	11																
10-6-76	11		11	11	7	7	7	7	11	11	11	11									5	4	5	5				
16-6-76	11	11	11	11	7	7	7	7	12	11	11	11									5	5	5	5				
24-6-76	11	11	11	11	8	8	8	8		12	12	12									7	8	7	7				
1-7-76	12	12	12	12	9	9	9	9													8	8	8	9				
8-7-76					10	10	10	10													8	8	8	9				
15-7-76					11	11	10	11													8	8	8	9				
22-7-76					11	11	11	11													10	10	10	10				
29-7-76					11	11	11	11													11	11	11	11				
5-8-76					12	12	12	12													11	11	11	11				
12-8-76																					11	11	11	11				
19-8-76																					12	12	12	12				
26-8-76																												
3-9-76																												
9-9-76																												
11-4-77					1	1	1	1					5	5	5	5	--	--	--	--					1	1	1	1
19-4-77					1	1	1	1					7	7	7	7	7	7	7	7					1	1	1	1
25-4-77					3	3	3	3					8	8	8	8	8	8	8	8					2	2	2	2
2-5-77					3	3	3	3					9	9	9	9	8	8	8	8					3	3	3	3
9-5-77					5	5	5	5					10	10	10	10	9	9	9	9					--	--	--	--
16-5-77					6	6	6	6					10	10	10	10	9	9	9	9					3	3	3	3
24-5-77					7	7	7	7					10	10	10	10	10	10	10	10					8	8	8	8
7-6-77					8	8	8	8					11	11	11	11	11	11	11	11					8	8	8	8
15-6-77					8	8	8	8					12	12	12	12	12	12	12	12					8	8	8	8
24-6-77					10	10	10	10																	--	--	--	--
30-6-77					10	10	10	10																	11	11	11	11
7-7-77					11	11	11	11																				
22-7-77					12	12	12	12																				
6-8-77																												
12-8-77																												
18-8-77																												
25-8-77																												
1-9-77																												
8-9-77																												
15-9-77																												
22-9-77																												

TABLE 13.2. (CONTINUED)

Date	*Poa secunda*				*Psoralea argophylla*				*Sphaeralcea coccinea*				*Stipa comata*				*Stipa viridula*				*Taraxacum officinale*				*Tragopogon dubius*				*Vicia americana*			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
18-3-76	3	3	3	3									1	1	1	1					1	1	1	1	2		2	2				
29-3-76	3	3	3	3									1	1	1	1	1				2	2	2	2	2	2	2	2				
12-4-76	3	3	3	3																	5	5	5	5	3	3	3	3				
19-4-76																					5	6	6	6	3	3	3					
2-5-76																					7	7	7	7								
10-5-76	5	5	5	5																	8	8	9	8								
15-5-76	6	6	6	6									3	3	3	3	2		2		10	10	10	10	5		5	5		8		
26-5-76	6	6	6	6	3.				3	3	3	3	3	3	3	3					10	10	11	11	5	5	5	5	7	7	8	8
2-6-76	7	7	7	7					4	4	4	4	3	3	3	3	4		4		11	11	11	11	6	6	6	6				8
10-6-76	8	8	8	8	4	4	4	4	5	5	5	5	7	7	8	7	6	6	6	6	11	11	11	11	7	7	7	7	11			
16-6-76	8	8	8	8	5	4	4	4	5	5	5	5	8	8	8	7	6	6	6	6	11	11	11	11	8	8	8	8				
24-6-76	9	9	9	9	5	5	6	5	9	9	9	9	9	9	9	9	7			7	11	11	11	11	8	8	8	9	9			11
1-7-76	11	11	11	11	9	9	9	9	9	9	9	9	10	10	10	10	9	9	9	9	11	11	11	11	10	10	10	10	11	11	11	11
8-7-76	11	11	11	11	10	10	10	10	9	9	9	9	10	10	10	10	11	10	10	10	11	11	11	11	10	10	10	10	11	11	11	11
15-7-76	12	12	12	12	10	10	10	10	9	9	10	10	11	11	11	11	11	10	10	10	11	11	11	11	10	10	10	10	11	11	11	11
22-7-76					10	10	10	10	10	11	10	11	11	11	11	11	11	11	11	11	11	11	11	11	10	10	10	10	11	11	11	11
29-7-76					10	10	10	10	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	10	11	11	11	11	11	11	11
5-8-76					10	10	10	10	11	11	11	11	12	12	12	12	11	11	11	11	11	11	11	11	10	11	11	11	12	12	12	12
12-8-76					11	11	11	11	11	11	11	11					12	12	12	12	12	12	12	12	11	11	11	11				
19-8-76					11	11	11	11	12	12	12	12													12	12	12	12				
26-8-76																																
3-9-76						12	11																									
9-9-76							12						1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
													1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	1	1	1	1
11-4-77	2	2	2	2									2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
19-4-77	2	2	2	2									--	--	--	--	--	--	--	--	8	8	8	8	3	3	3	3	--	--	--	--
25-4-77	3	3	3	3									4	4	4	4	3	3	3	3	9	9	9	9	3	3	3	3	8	8	8	8
2-5-77	--	--	--	--									4	4	4	4	--	--	--	--	10	10	10	10	4	4	4	4	8	8	8	8
9-5-77	5	7	7	7					4	4	4	4	4	4	4	4	--	--	--	--	11	11	11	11	4	4	4	4	8	8	8	8
16-5-77	6	6	6	6					5	5	5	5	6	6	6	6	6	6	6	6	11	11	11	11	9	9	9	9	8	8	8	8
24-5-77	8	8	8	8					5	5	5	5	7	7	7	7	6	6	6	6	12	12	12	12	9	9	9	9	9	9	9	9
7-6-77	8	8	8	8					6	6	6	6	9	9	9	9	9	9	9	9	12	12	12	12	10	10	10	10	10	10	10	10
15-6-77	8	8	8	8					8	8	8	8	10	10	9	10	10	10	10	10					10	10	10	10	--	10	--	--
24-6-77	10	10	10	10					9	9	9	9	10	10	10	--	10	10	10	--					11	11	11	--	10	10	10	--
30-6-77	11	11	11	11					9	9	9	9	11	11	11	11	11	11	11	11					12	12	12	12	12	12	12	12
7-7-77									10	10	10	10	12	12	12	12	12	12	12	12												
22-7-77									11	11	11	11																				
6-8-77									12	12	12	12																				
12-8-77																																
18-8-77																																
25-8-77																																
1-9-77																																
8-9-77																																
15-9-77																																
22-9-77																																

TABLE 13.3. CHEMICAL CONSTITUENTS OF LIVE *AGROPYRON SMITHII* AND *KOELERIA CRISTATA*

Site	Date	Ash (%) Rep. 1	Ash (%) Rep. 2	Nitrogen (%) Rep. 1	Nitrogen (%) Rep. 2	Phosphorus (%) Rep. 1	Phosphorus (%) Rep. 2	Sulfur (%) Rep. 1	Sulfur (%) Rep. 2
				Agropyron smithii					
ZAPS I, Control	1 June 1975	7.6	7.7	1.9	1.8	.22	.23	.11	.08
	1 July 1975	6.7	6.9	1.3	1.3	.14	.14	.09	.10
	1 August 1975	7.8	7.3	1.1	1.1	.12	.10	.13	.10
	17 May 1976	6.9	6.9	2.3	2.2			.05	.09
	15 June 1976	6.3	6.7	1.6	1.5			.09	.09
	10 July 1976	6.8	6.4	1.3	1.2			.10	.09
	9 August 1976	6.5	7.3	1.0	0.9			.08	.09
	17 September 1976	7.0	7.0	0.8	0.7			.12	.09
ZAPS I, Low	1 May 1975	8.3	8.7	2.6	2.4	.27	.22	.13	.13
	1 June 1975	8.1	7.5	1.6	1.8	.23	.22	.15	.14
	1 July 1975	6.4	6.3	1.3	1.2	.15	.16	.14	.13
	1 August 1975	7.8	6.7	1.1	1.0	.16	.20	.14	.13
	17 May 1976	7.2	6.9	2.2	2.1			.15	.07
	16 June 1976	7.6	7.2	1.3	1.4			.14	.13
	11 July 1976	7.5	6.4	1.1	1.0			.18	.13
	10 August 1976	8.0	7.7	0.8	0.8			.10	.20
	17 September 1976	8.1	7.5	0.6	0.6			.18	.17
ZAPS I, Medium	17 May 1975	8.4		2.7		.36		.14	
	1 June 1975	8.1	8.0	1.5	1.8	.20	.26	.23	.19
	1 July 1975	8.1	7.2	1.3	1.4	.18	.15	.25	.23
	1 August 1975	8.1	7.5	1.2	1.0	.15	.13	.24	.28

TABLE 13.3. CONTINUED

Site	Date	Ash (%)		Nitrogen (%)		Phosphorus (%)		Sulfur (%)	
		Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
		Agropyron smithii (cont.)							
ZAPS I, Medium (cont.)	18 May 1976	7.7	6.7	2.0	2.1			.16	.16
	18 June 1976	7.2	7.0	1.4	1.5			.22	.21
	12 July 1976	6.9	7.1	1.1	1.1			.23	.21
	11 August 1976	8.7	7.7	1.0	0.9			.31	.27
	17 September 1976	8.7	7.9	0.6	0.6			.25	.26
ZAPS I, High	17 May 1975	8.3	8.3	2.6	2.7	.31	.31	.13	.13
	1 June 1975	7.9	8.2	2.0	1.9	.23	.26	.21	.24
	1 July 1975	8.4	8.2	1.2	1.2	.18	.17	.27	.28
	1 August 1975	10.1	7.1	1.0	1.0	.12	.12	.31	.35
	19 May 1976	8.7	7.8	1.9	1.9			.25	.22
	19 June 1976	8.4	7.4	1.3	1.3			.23	.25
	13 July 1976	8.5	8.1	1.1	1.0			.35	.30
	12 August 1976	9.1	9.8	0.9	0.9			.42	.40
	17 September 1976	9.4	9.2	0.7	0.7			.37	.35
ZAPS II, Control	21 May 1976	9.2	8.8	2.2	2.3			.14	.12
	20 June 1976	7.6	7.7	0.3	1.9			.09	.12
	14 July 1976	8.9	8.3	1.3	1.4			.09	.10
	14 August 1976	8.6	8.0	1.0	1.1			.11	.09
ZAPS II, Low	22 May 1976	8.4	8.1	2.5	2.4			.12	.16
	21 June 1976	6.5	6.4	2.0	1.8			.12	.13
	15 July 1976	8.1	8.7	1.5	1.7			.16	.17
	13 August 1976	7.7	7.1	1.1	1.1			.15	.15
	18 September 1976	8.5	8.9	0.9	1.0			.16	.15

TABLE 13.3. CONTINUED

Site	Date	Ash (%)		Nitrogen (%)		Phosphorus (%)		Sulfur (%)	
		Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
		Agropyron smithii (cont.)							
ZAPS II, Medium	23 May 1976	10.1	9.0	2.3	2.2			.23	.19
	22 June 1976	8.1	8.0	1.6	1.6			.23	.23
	16 July 1976	9.2	8.8	1.3	1.3			.27	.27
	15 August 1976	9.5	8.5	1.0	1.0			.25	.31
	18 September 1976		8.6		0.8				
ZAPS II, High	24 May 1976	9.4	9.4	2.4	2.6			.30	.34
	23 June 1976	8.1	8.2	1.7	2.1			.31	.39
	17 July 1976	9.5	8.8	1.4	1.5			.45	.41
	16 August 1976	9.7	9.0	1.2	1.2			.52	.44
	18 September 1976		10.5		1.3				.35
		Koeleria cristata							
ZAPS I, Control	1 June 1975	10.6	10.3	1.8	1.6	.20	.25	.10	.13
	1 July 1975	9.8		1.3		.19		.09	
	17 May 1976	8.7	8.4	1.8	1.7			.06	.11
	15 June 1976	10.0	10.3	1.3	1.3			.11	.07
	10 July 1976	10.0	10.0	1.2	1.1			.08	.08
	9 August 1976	10.0	9.7	1.3	1.1			.08	.07
ZAPS I, Low	1 July 1975	9.9	9.5	1.3	1.2	.20	.20	.15	.14
	17 May 1976	8.8	9.2	1.7	1.6			.08	.09
	16 June 1976	9.9	9.5	1.4	1.3			.14	.12
	11 July 1976	10.1	10.0	1.1	1.1			.16	.15
	10 August 1976	10.3	10.6	1.1	1.0			.18	.18

TABLE 13.3. CONTINUED

Site	Date	Ash (%)		Nitrogen (%)		Phosphorus (%)		Sulfur (%)	
		Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
		Koeleria cristata (cont.)							
ZAPS I, Medium	1 June 1975	10.5		1.7		.24		.13	
	17 May 1976	10.0	10.0	1.7	1.7			.17	.18
	18 June 1976	10.4	9.5	1.3	1.3			.21	.21
	12 July 1976	11.6		1.1				.23	
	11 August 1976	10.7	10.5	1.2	1.1			.25	.25
	17 September 1976	11.3	10.0	0.9	0.9			.10	.20
ZAPS I, High	1 July 1975		12.3		1.4		.22		.39
	19 May 1976	10.4	10.5	1.8	1.6			.21	.22
	19 June 1976	12.4	11.3	1.4	1.3			.28	.21
	13 July 1976	12.1	12.6	1.1	1.0			.30	.27
	12 August 1976	13.4	12.3	1.2	1.2			.37	.39
ZAPS II, Control	20 June 1976	13.3		1.3				.07	
	14 July 1976		12.6		1.3				.23
	14 August 1976		10.2		1.4				.06
	18 September 1976	11.1		1.0					
ZAPS II, Low	21 June 1976		9.4		1.3				.14
	13 August 1976	10.3		1.4				.10	

TABLE 13.3. CONTINUED

Site	Date	Ash (%)		Nitrogen (%)		Phosphorus (%)		Sulfur (%)	
		Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
		Koeleria cristata (cont.)							
ZAPS II, Medium	23 May 1976	12.0	10.7	1.9	1.8			.21	.21
	22 June 1976	10.7	11.0	1.3	1.3			.20	.26
	12 July 1976	12.2	12.0	1.3	1.3			.24	.22
	15 August 1976	10.4	9.8	1.3	1.3			.27	.27
	18 September 1976		12.2		1.1				.18
ZAPS II, High	24 May 1976	10.3	11.3	2.1	1.8			.27	.23
	23 June 1976	12.4	13.2	1.4	1.4			.26	.28
	17 July 1976	11.5	11.8	1.3	1.5			.33	.32
	16 August 1976	11.1	10.8	1.5	1.5			.39	.35

TABLE 13.4. CHEMICAL CONSTITUENTS OF LIVE *AGROPYRON SMITHII* AND *KOELERIA CRISTATA*

Site	Date	T.A.C. (% wt)		Mg (mg kg^{-1})		Fl (mg kg^{-1})	
		Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
		Agropyron smithii					
ZAPS I, Control	17 May 1975	12.0	13.5	.14	.12		
	1 June 1975	7.4		.18		2.1	2.4
	1 July 1975	12.0	12.0	.20	.15	0.7	1.0
	1 August 1975	13.0	12.0	.19	.17	1.5	1.5
	17 May 1976	10.7	10.8				
	15 June 1976	10.8	11.7				
	10 July 1976	12.4	15.2				
	9 August 1976	13.7	15.1				
	17 September 1976	10.7	12.0				
ZAPS I, Low	17 May 1975	9.7	11.0	.15	.14	1.4	1.4
	1 June 1975	6.6	9.0	.15	.15	1.9	0.9
	1 July 1975	11.0	11.0	.13	.10		0.9
	1 August 1975	13.0	14.0	.15	.12	1.3	1.0
	17 May 1976	9.2	10.9				
	16 June 1976	10.7	9.4				
	11 July 1976	14.4	14.7				
	10 August 1976	15.5	15.7				
	17 September 1976	13.3	11.4				
ZAPS I, Medium	17 May 1975	12.0		.13		1.2	
	1 June 1975	5.9	5.6	.14	.13	1.0	
	1 July 1975	8.5	9.5	.16	.11		1.3
	1 August 1975	12.0	12.0	.12	.15	2.0	1.8

TABLE 13.4. CONTINUED

Site	Date	T.A.C. (% wt) Rep. 1	T.A.C. (% wt) Rep. 2	Mg (mg kg^{-1}) Rep. 1	Mg (mg kg^{-1}) Rep. 2	Fl (mg kg^{-1}) Rep. 1	Fl (mg kg^{-1}) Rep. 2
Agropyron smithii (cont.)							
ZAPS I, Medium (cont.)	18 May 1976	8.3	8.4				
	18 June 1976	9.5	10.5				
	12 July 1976	11.9	12.3				
	11 August 1976	12.0	14.2				
	17 September 1976	10.9	13.6				
ZAPS I, High	17 May 1975	11.0		.15			2.1
	1 June 1975	8.5	9.8	.13	.14	2.0	1.8
	1 July 1975	8.1	9.4	.11	.14	1.9	1.6
	1 August 1975	11.0	11.0	.13	.14	1.1	1.8
	20 May 1976	11.6	10.4				
	19 June 1976	11.3	11.2				
	13 July 1976	9.7	10.9				
	12 August 1976	11.4	11.9				
	17 September 1976	12.3	12.5				
ZAPS II, Control	21 May 1976	7.7	8.9				
	20 June 1976	12.6	13.3				
	14 July 1976	12.2	11.2				
	14 August 1976	12.1	11.5				
ZAPS II, Low	22 May 1976	7.6	7.3				
	21 June 1976	12.2	13.0				
	15 July 1976	10.5	9.7				
	13 August 1976	12.4	11.8				
	18 September 1976	11.8	12.0				

TABLE 13.4. CONTINUED

Site	Date	T.A.C. (% wt)		Mg (mg kg^{-1})		Fl (mg kg^{-1})	
		Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
	Agropyron smithii (cont.)						
ZAPS II, Medium	23 May 1976	9.7	8.8				
	22 June 1976	8.2	8.5				
	16 July 1976	10.2	10.9				
	15 August 1976	10.8	12.4				
	18 September 1976		9.7				
ZAPS II, High	24 May 1976	8.1	8.9				
	23 June 1976	8.9	9.6				
	17 July 1976	8.3	10.0				
	16 August 1976	9.4	10.7				
	18 September 1976		8.7				
	Koeleria cristata						
ZAPS I, Control	17 May 1975	13.0	12.0	.15	.15		
	1 June 1975	[illegible].2	6.8	.18	.19		1.9
	1 July 1975	11.0	11.0	.17	.17		1.8
	1 August 1975	10.0	13.0	.22	.18		
	17 May 1976	19.9	14.1				
	15 June 1976	9.6	10.5				
	10 July 1976	9.2	10.8				
	9 August 1976	14.1	12.2				

TABLE 13.4. CONTINUED

Site	Date	T.A.C. (% wt) Rep. 1	T.A.C. (% wt) Rep. 2	Mg (mg kg^{-1}) Rep. 1	Mg (mg kg^{-1}) Rep. 2	Fl (mg kg^{-1}) Rep. 1	Fl (mg kg^{-1}) Rep. 2
		Koeleria cristata (cont.)					
ZAPS I, Low	1 May 1975	13.0	11.0	.16	.16	3.8	2.8
	1 June 1975	7.7	6.2	.18	.16		
	1 July 1975	8.9	8.8	.16	.14	1.6	2.0
	1 August 1975	14.0	14.0	.19	.18		1.3
	17 May 1976	12.0	14.0				
	16 June 1976	9.6	9.7				
	11 July 1976	10.3	12.6				
	10 August 1976	13.7	15.6				
ZAPS I, Medium	17 May 1975	13.0	12.0	.17	.18		
	1 June 1975	11.0	7.5	.13	.17		
	1 July 1975	8.9	8.6	.15	.16	2.8	1.2
	1 August 1975	13.0	14.0	.16	.19		
	17 May 1976	10.4	12.6				
	18 June 1976	10.9	10.5				
	12 July 1976	9.6					
	11 August 1976	12.6	13.5				
	17 September 1976	11.4	10.5				
ZAPS I, High	17 May 1975	10.0	13.0	.17	.16		
	1 June 1975	8.1	7.5	.16	.16		
	1 July 1975	8.7	7.7	.15	.18		
	1 August 1975	11.0	11.0	.17	.18		

TABLE 13.4. CONTINUED

Site	Date	T.A.C. (% wt)		Mg (mg kg^{-1})		Fl (mg kg^{-1})	
		Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
		Koeleria cristata (cont.)					
ZAPS I, High (cont.)	19 May 1976	10.9	12.5				
	18 June 1976	6.8	9.5				
	13 July 1976	6.0	7.7				
	12 August 1976	10.1	10.8				
ZAPS II, Control	20 June 1976	11.3					
	14 July 1976		6.6				
	14 August 1976		15.3				
ZAPS II, Low	21 June 1976		15.5				
	13 August 1976	15.1					
ZAPS II, Medium	22 May 1976	9.2	10.2				
	22 June 1976	9.1	8.7				
	12 July 1976	9.9	9.9				
	15 August 1976	14.7	14.5				
ZAPS II, High	24 May 1976	10.3	10.4				
	23 June 1976	6.6	8.4				
	17 July 1976	8.5	8.9				
	16 August 1976	11.9	13.9				

SECTION 14

THE EFFECTS OF "LOW LEVEL SO_2" EXPOSURE ON SULFUR ACCUMULATION AND VARIOUS PLANT LIFE RESPONSES OF SOME MAJOR GRASSLAND SPECIES ON THE ZAPS SITES

P. M. Rice, L. H. Pye, R. Boldi, J. O'Loughlin
P. C. Tourangeau, C. C. Gordon

ABSTRACT

Organisms on the ZAPS sites accumulated total sulfur in a gradient which paralleled ambient SO_2 concentration. However, significant edge effects occurred on the treatment plots. Experimental designs to detect biological responses should consider this source of variance. Accumulation, partitioning, and cycling of sulfur was studied in six prairie vegetation species. A peak of sulfur in live tissues during greenup periods was masked by subsequent fumigation. Sulfur in plants increased as a function of ambient SO_2 concentration and time over the active growth period. Plants of similar growth habit and phenological timing accumulated sulfur at similar rates. Species specific differences in sulfur partitioning were found, *A. smithii* dead shoots acting as an apparent sink. Sulfur in roots of perennial grasses correlated positively with shoot sulfur. Sulfur inputed during previous years appeared in live shoots during subsequent years' growth. This carry over of sulfur is termed the residual sulfur effect. Data from two sites with an 80-year fumigation history indicated that the residual sulfur effect causes a cumulative increase in biologically incorporated sulfur. Rainfall can remove 13 percent of the sulfur held by leaves, but storm size is biased towards low volume rainfall events that may actually increase SO_2 scavenging. Overwintering of shoots releases 69 percent of their sulfur content. Ground sampling could not confirm aerially delineated patterns of SO_2 drift off the treatment plots. Premature leaf senescence was associated with increasing SO_2, while leaf initiation rates were generally unaffected. Increasing variance in fluoride content suggests a disruption of normal root uptake processes. Seed germination capacity, speed of germination, and seed weight were depressed on the higher treatment plots in several perennial range grasses. The presence of an endomycorrhizal phycomycete in *A. smithii* was reported. Population levels of the beneficial associate declined with SO_2 treatment. Histological preparation indicates that the normal morphology of fungi was disrupted at low SO_2 treatment levels with an alteration of the symbiosis to a potentially pathologic state.

INTRODUCTION

Vegetation Studies

Opinions about the levels of SO_2 which can damage vegetation are probably as numerous as published fumigation studies and field studies. Most theories were derived either from field studies in areas subject to acute SO_2 plant damage (such as Duck Town, Tennessee; Sudbury, Ontario; Trail, British Columbia, and Anaconda, Montana) or from studies of pollution levels and effects in cities monitored by the National Air Pollution Control Agency during the 1960s and reported in the EPA SO_2 criteria document. Sulfur dioxide effects have also been studied in exacting controlled fumigation studies by numerous investigators examining the anatomical or physiological impacts of SO_2 dosages at various durations on a variety of plant species. Because the individuals actually conducting field or controlled fumigation studies have reported diverse and sometimes opposing results, researchers from federal and state agencies, industries, universities, and private laboratories have tried to devise more sophisticated methodologies to determine the potential and real impacts of SO_2 upon plant species and/or plant communities. Current sophistication in controlled SO_2 fumigation studies is apparent in the open top field chamber studies and the wind tunnel fumigation studies by Ashenden and Mansfield (1977).

The EPA-CERL controlled fumigation studies conducted during the last three years in the Custer National Forest of southeastern Montana represent the first attempt by researchers to determine SO_2 effects in an established grassland ecosystem. This area of Montana is particularly suitable for such an open field experiment because it is pristine and has received little or no measurable SO_2 or fluoride contamination in the past (Northern Cheyenne Tribe, 1976; Gordon *et al.*, 1976). This study is also of immense importance to the farming and ranching communities and Indian tribes of southeastern Montana; during the next decade, southeastern Montana, as well as northern Wyoming, may become the site of large stationary SO_2 sources, including coal-fired power plants and gasification or liquefaction industries.

Currently, there is a tremendous paucity of literature concerning the potential impacts of SO_2 and all other phytotoxic gases upon most of the flora and fauna species of this agricultural land. Even more serious is the lack of adequate data on the interrelationships between species of this grassland; the potential indirect impacts of SO_2 damage on one or two species will be difficult to detect or quantify in this relatively short study.

While the ZAPS study is the most extended controlled fumigation study reported in the literature, it represents only a fraction of the time that field researchers in the United States, Canada, and Europe have spent attempting to document the impacts of SO_2 pollution on the ecosystems surrounding large stationary emissions sources. Furthermore, we believe that ecosystems are not damaged or destroyed in the first decade of pollution but rather gradually over a longer period of operation of that source, as in Anaconda, Montana.

We caution the reader to remember that the impacts and lack of impacts reported and discussed herein occurred during a very short period of time in the evolution of this grassland ecosystem. We are further restricted by the uncertainties inherent to known field methodologies and our own lack of understanding of the interrelationships among the species of this ecosystem.

During 1976 and 1977, our ZAPS studies included a substantial amount of sulfur analyses of plant foliage and root tissues from the different treatment plots. Because it has long been known that chronic exposure to SO_2 fumigation causes excessive sulfur accumulation in the foliage of plant species, we hypothesized that the grass, forb, and shrub species fumigated at the different treatment levels would accumulate different levels of sulfur over the growing season. While this may seem an easily-satisfied hypothesis, it was important that it be tested because the baseline sulfur levels of all the plant species on the ZAPS plots were not known.

Numerous researchers have conducted "long-term" fumigation studies that did not include determination of pre- and post-fumigation sulfur levels in the plant tissue (Booth *et al.*, 1976; Bleasdale, 1973; Cocking, 1973; Hill *et al.*, 1974; Ashenden and Mansfield, 1977; Section 15, etc.). If the seasonal baseline or normal sulfur concentrations in plant tissues of unfertilized, indigenous species are established before this vegetation is fumigated or impacted by chronic levels of SO_2, investigators could determine subsequent sulfur accumulation and any related morphological or physiological damage. There have never been any studies to determine a possible relationship between excessive sulfur accumulation and vegetative or reproductive structure damage.

Our studies of vegetation collected at the ZAPS sites, as well as from our ponderosa pine/skunkbush sites and the EPA exclosure sites on the McRae and Kluver ranches, indicate that sulfur accumulation can be determined in plant foliage exposed to chronic SO_2 pollution episodes. While there are seasonal variations of sulfur levels in foliage from the pristine lands of southeastern Montana, this variability should not interfere with the usefulness of these concentrations as indicators of SO_2 fumigation or trespass.

We have been studying the possibility that root tissues of perennial grasses at the ZAPS sites contain residual sulfur. If portions of the sulfur taken in by the foliage are transported to the root tissue during or after the growing season, some of this sulfur might be returned to leaf tissues during the next growing season; biologically-incorporated sulfur may also continue to increase over long periods of time (five to ten years).

Our studies of sulfur in western wheatgrass roots collected on the ZAPS sites, as well as in Anaconda, Montana, indicate that this residual sulfur may play an extremely important role in long-term chronic air pollution impacts. In the spring of 1977, soil from the Anaconda area was transported to the University of Montana botany gardens, and four species of plants common on the ZAPS sites were established in this soil. Studies on residual sulfur in the foliage and roots of these four species will be conducted during the 1978 growing season and reported in the Fifth Interim Report.

We are continuing our work on viability and germination rates of seeds collected from the different SO_2 treatment levels on ZAPS I and II. Seed viability and germination studies of samples collected during the 1977 growing season will commence in May of this year, and the results will be included in the Fifth Interim Report. Thus far, our survey of the literature has not revealed any similar studies of fumigated grass and forb species.

During 1976, we established a plot with plant communities similar to those on the ZAPS sites. This Off Plot Control (OPC) is located about 2 km from the ZAPS to protect it from any fugitive SO_2. It was our hypothesis that if fugitive SO_2 from the treatment plots was drifting across the ZAPS IA and IIA plots and impacting the vegetation, data obtained from the OPC would help us ascertain the impacts of the fugitive gas. Because the grassland ecosystems on the ZAPS sites were pristine before the commencement of this fumigation study, we feel that a true comparison between the vegetation fumigated by the different delivery treatments will be difficult to ascertain when the control plots are also being fumigated.

Mychorrhizal Studies*

No quantitative analyses of vesicular-arbusicular (VA) or endomycorrhizal (EDM) populations within native U.S. grasslands have ever been conducted, nor have the effects of SO_2 fumigation on endomycorrhizal populations ever been studied. Reasons for the lack of such investigations are the necessary expertise required and the tedious methodology that must be employed. Such methodologies have lagged far behind the increasing importance that is currently attributed to mycorrhizae (M) and their associated symbionts and hosts.

EDM are ubiquitous in their association with angiosperms, their alliance including members of the largest and economically most important crop species belonging to the Gramineae and Leguminosae. Additional phyla known to be mycorrhizal are Bryophyta (especially Hepaticae), Pteridophyta and Coniferophyta (Ainsworth and Sussman, 1968). Ectomycorrhizae (ECM), differentiated from EDM by the former's formation of Hartig nets and their failure to penetrate hosts intracellularly, are found on most woody angiosperms and gymnosperms and gymnosperms such as *Pinus* spp. and *Picea* spp. Endophytes of ECM typically belong to the class Basidiomycetes, while those of EDM are thought to belong to class Phycomycetes. A recent monograph (Gerdemann and Trappe, 1973) describes the taxonomy, occurrence, and distribution of EDM endophytes which, for the most part, belong to the family Endogonaceae.

---

*This study was primarily carried out Laurel Pye, a graduate student in the Botany Department at the University of Montana. Field studies continuing through 1978 will be reported in the Fifth Interim Report.

The mycorrhizal relationship is a dynamic symbiotic biochemical-physiological interaction. The presence of mycorrhizae can generally be assumed because they not only date back to the Devonian but are more often present on plants than not (Baylis, 1972a, 1974). Although mycorrhizae are usually considered beneficial and symbiotic, the mycorrhizal relationship has also been labeled "reciprocally parasitic" (Hacskaylo, 1972) and "potentially pathogenic" (Harley, 1959). While some have argued that mycorrhizal infestation represents the end product of specialized parasitism (Garrett, 1956), others reject this idea and maintain that "there seems no need to assume that such conditions need not only arise from a long period of evolution, or that parasites must always evolve from avirulence to symbioses" (Bawden, 1957).

Only in the past two decades has the mycorrhizal condition taken on significance and become a matter of serious scientific investigation. This increased concern resulted from both the ubiquity of mycorrhizae in natural habitats and the disturbance of such through man's manipulation of the environment.

Phosphorous is among the most common and important elements in a plant, its distribution being always roughly parallel to that of protein. Of equal importance is the highly reactive, complex, organic, cation-binding compound phytate, otherwise known as phytic acid or inositol hexaphosphate. It is in this form that phosphorous is most often found both within plants and the organic humus layer of soil (Russell, 1973).

Because mycorrhizae can very efficiently transport phosphorous from the humus layer to the plant, most plants are mycorrhizal. Mycorrhizae have evolved as an integral part of a system by which a plant secures sufficient phosphorous, lack of which most often limits plant growth (Baylis, 1972b). In a detailed review of the literature, Mosse (1973) stated, "results confirm that mycorrhizal roots take up more phosphate than non-mycorrhizal roots, that it is translocated to the hosts' shoots . . . and that the striking effects of inoculation on plant growth and phosphate uptake have been demonstrated beyond a doubt."

Experiments have shown that mycorrhizal roots are capable of absorbing 3 to 16 times as much available organic phosphorous as non-mycorrhizal roots (Sanders and Tinker, 1973). Similar results have been reported by Baylis (1967), Ross (1971), Gerdemann (1964, 1968), Gray and Gerdemann (1969, 1973), Voigt (1969), Murdoch *et al.* (1967), Jackson *et al.* (1972), Sanni (1976a, 1976b), and Sanders *et al.* (1977). In addition, zinc deficiency symptoms in peach and stunted growth in citrus have been related to inadequate nutrition in non-mycorrhizal soils (Gilmore, 1971; Kleinschmidt and Gerdemann, 1972).

Because of the efficiency with which they take up phosphorous and associated cations, mycorrhizae also stimulate: (1) flower production in petunia, (2) formation of fruit in strawberry, (3) development of pollen in maize, and (4) the amount of vascular tissue in all of these plants (Daft and Okusanya, 1973). Mycorrhizal infection in cotton has also been correlated with earlier flowering and boll maturation (Rich and Bird, 1974), while infection in tomato has been reported to stimulate ontogeny and to delay leaf senescence (Daft and Nicolson, 1969). Furthermore, mycorrhizae have been found to strongly stimulate both growth and nodulation of legumes and the persistence of anaerobic, nitrogen-fixing bacteria such as Azotobacter and Clostridium (Richards and Voigt, 1964; Daft and El-Giahmi, 1974; Crush, 1974; Mosse, 1977). The enhancement of hormone levels by mycorrhizae has been implicated as the cause of many such symptoms. The production of growth-promoting cytokinins, such as Zeatin and Zeatin riboside, have been demonstrated in the case of the ECM endophyte *Rhizopogon roseolus* (Miller, 1967).

Several experimenters have demonstrated that current fertilization practices, whereby soil fertility levels are greatly augmented with superphosphates, have a depressing effect on mycorrhizal levels (Daft and Nicolson, 1966; Ross, 1971; Khan, 1972; Mosse, 1973). In the case of soybeans, for example, "phosphorous concentration in foliage of mycorrhizal plants at the lowest phosphate fertilization level was found to be greater than that of nonmycorrhizal plants at the highest phosphate level" (Ross, 1971). The effect probably is a disruption of the symbiotic state due to a change in host plant metabolism rather than a direct inhibition of fungal growth (Mosse, 1973).

The ability of air pollutants, such as SO_2, to alter plant metabolism or disrupt the symbiotic state as exhibited by lichens is well-documented. Whether SO_2 similarly disrupts the symbiotic mycorrhizal state is an important and yet unanswered question. Before one can consider the impact SO_2 may have on mycorrhizae, one must consider those factors, besides phosphorous, which "naturally" determine infection levels.

Nicolson (1960) has stressed the fact that mycorrhizal infection levels are influenced primarily by the degree of habitat stability and the amount of undecomposed organic matter present. He found high levels of infection in plant communities that were stable but did not have high levels of organic matter; large amounts of organic matter can stimulate microbial activity which eventually may damage the endophytes. In a European grassland study, no consistent change of infection was found to occur with change in sub-surface geology or soil pH, but highest infection levels occurred in the shallowest soils (Read *et al.*, 1976). These findings suggested to the authors that nutrient levels or water stress might determine levels of infection. The same authors have also suggested that high infection levels indicate an intensely competitive situation whereby the nutrient status of an environment is depleted and the resistance of the host to infection is thus reduced.

Recent experiments have revealed that mycorrhizal infection and root competition combine to produce a result quite different than the effect of each factor separately. Trinick (1977) found growth of red clover stimulates infection in *Lupinus cosentrini* Gus. *Holcus lanatus* L. was found to have only a slight advantage in yield over *Lolium perenne* L. when singularly subjected to root competition or mycorrhizal infection, while the combination resulted in considerable depression of *Lolium perenne* L. (Fitter, 1977).

Any determination of infection, however, must be reviewed with discretion because conflicting results have been reported. Mejstrik (1972) found that soil factors which reduce host growth, such as soil nutrient levels, also reduce frequency and intensity of mycorrhizal development; therefore, a host plant "weakened" by low nutrient levels is not necessarily subject to a higher level of infection as was suggested earlier. Soil fertility levels in Spain also had little effect on mycorrhizae populations (Hayman et al., 1976).

In addition, one must take into account experimenters' methodology in determining root infection levels. Mosse (1973) reported that spore numbers may or may not be significantly correlated with root infection. Amounts of external mycelium also cannot always be correlated with root infection levels (Nicolson, 1960). Finally, one cannot liberally interpret the results of any "artificial" glasshouse or potculture experiment, unless the ecological or competitive status of the host in a plant community is accounted for.

Most papers published on sulfur and its effect on fungi describe sulfur as a fungicide, a fact recorded by early classical writers (Ainsworth and Sussman, 1965). Although sulfur, in the form of elemental sulfur or lime sulfur (calcium polysulfide and calcium thiosulfate) is fatal to numerous fungal species, particularly powdery mildews, it is fatal only to a limited range of the higher plants (Marten, 1959; Ainsworth and Sussman, 1965).

Experiments have been conducted in which sulfur was supplied as magnesium sulfate, dilute $H_2S^{35}O_4$ and $S^{35}O_4^{=}$ to potted and inoculated host plants. Results indicated the following: (1) In the case of red clover (*Trifolium pratense* L.) and maize (*Zea mays* L.), mycorrhizal roots take up sulfur more efficiently than non-mycorrhizal roots (Gray and Gerdemann, 1973), and (2) in the case of *Pinus radiata* ECM, "the bulk of sulfur destined for the shoot of the host is not metabolized by the fungus but has a more direct pathway through the mycelium from ambient solution to host roots, whereupon it is transported to host shoots and then progressively accumulated by shoot apices from older leaves" (Biddulph *et al.*, 1958; Morrison, 1962). Heagle (1973) noted a study by Sobotka (1964) in which the impact of SO_2 on mycorrhizae was examined under field conditions. He found the ECM associated with spruce to be abnormal in an SO_2-polluted area.

Variable responses to SO_2 have been recorded in several pathogenic fungi (Hibben, 1966; Couey, 1965). It is thought, however, that pollutants such as SO_2 are causal agents encouraging the spread of fungal disease in forests and crops (Grywacz, 1971; Heagle, 1973; Treshow, 1968, 1975). If air pollutants can bring about any one of the following conditions: (1) Change in external-internal host biochemistry; (2) alternation in quantity or quality of host or fungal exudates, or (3) cellular or organelle structural variation, then behavior modification, e.g., host recognition of pathogens or symbionts, is likely to occur.

Both ECM and EDM are thought to play a considerable role in disease deterrence (Zak, 1964; Harley, 1959; Marx, 1972; Slankis, 1974). Possible resistance factors are physical barriers created by the presence of mycorrhizae, exudates produced in conjunction with the symbiotic relationship, or merely the healthier state imparted by the relationship. The means and extent to which mycorrhizae serve in such a protective capacity are not yet clear. A recent study of the Cotton Stunt Disease Complex showed that damage to cotton by phytopathogenic nematodes is magnified when the symbiotic mycorrhizal state is impaired (Rich and Bird, 1974; Bird *et al.*, 1974).

The fungal endophyte which forms VA mycorrhizae has been identified in most cases as one of several species belonging to the family Endogonaceae (Mosse, 1973). All phases of their growth are quite unique and distinct in their appearance from other fungal infection types that might occur within a grass root. It is advisable in any case to examine specimens under both a binocular and light microscope to note the continuity which should occur between external and internal phases because profuse connections of this sort are not readily formed by *Pythium* spp. or other obligate parasites whose growth is normally restricted in soil. A few species, such as *Pythium* spp., are known to form VA type infections in rare instances (Ainsworth and Sussman, 1968).

It is also possible to discern the presence of mycorrhizae on grass roots because of their conspicuous sandy encasements. Such encasements often give roots a typical yellowish brown felty appearance. Nicolson (1959) and Harley (1959) published detailed descriptions of the grass endophyte; these descriptions are summarized in the following account.

Exterior hyphae are of two types. The first type is thick-walled with a diameter of 20μ to 27μ, aseptate, multinucleate, and filled with a dense cytoplasm and conspicuous oil globules. Such hyphae often run for a considerable distance along a root's length, branching at times to bear exterior vesicles or to pass into the soil or host root hairs. Branching from the latter are thin-walled hyphae with a diameter of 3μ to 5μ. These hyphae are considered to be ephemeral because after a period of active growth they become septate or shriveled and are easily lost from the main hyphae. This process causes many angular protrusions in the main hyphae. Several authors have attributed this "response" of the thin-walled hyphae to be "an inherent capability elicited when the hyphae are mechanically wounded or exposed to other types of deleterious or degenerative processes" (Gerdemann, 1955; Kinden and Brown, 1975).

Morley and Mosse (1976) described the appearance of abnormal hyphae. Such hyphae are characterized by "abortive" entry points, masses of irregular, vesicle-like swellings around functional entry points and short, distorted swollen or finger-like hyphae arising at the entry point from exceptionally large, irregularly-shaped hyphae.

Development of the endophyte is most profuse in the inner cortical area. Although one author observed what he thought to be mycorrhizae in the endodermis and pericycle (Shterenberg and Kostyak, 1967), most workers agree that mycorrhizae are never found in the endodermis, pericycle and enclosed vascular tissue, nor in the meristimatic region of actively growing root tips.

Kinden and Brown (1975) suggested that the actual penetration of a host cell wall by the endophyte is more than a strictly "mechanical" process and that it may well involve some type of enzyme degradation because cortical cells are neither ruptured nor damaged by the endophyte's presence. Their electron microscopy studies showed that a host consistently responds to the invading endophyte by depositing wall material which does not reduce the activity of the endophyte but confines or localizes it.

Penetration hyphae begin to disintegrate with the formation of arbuscules in inner cortical cells. Arbuscules are a highly branched terminal structure and are considered to be a complex haustorium (Cox and Sanders, 1974). When present, arbuscules appear as a dense granular mass in the lumen of a cortical cell. Arbuscular formation is usually greatest when the host is still in a vegetative state (Saif, 1977). Arbuscules are not permanent structures and invariably undergo disintegration or "digestion" by the host. An increase in host cytoplasmic volume and numbers of mitochondria and ribosomes has been shown to occur conjunctively with the deterioration process (Kinden and Brown, 1975). The authors suggested that this process releases substantial quantities of mineral nutrients which are absorbed by the host. Cox and Tinker (1976), however, think this is unlikely and that nutrient release to the host is accomplished by transfer across living fungal-host membranes.

Vesicle formation takes place after arbuscular appearance, but before their disintegration. Both internal and external vesicles can occur; they are considered to be analogous (Butler, 1939), although this is not unequivocally accepted (Mosse, 1973). External vesicles are borne either terminally or intercalary on hyphae and exhibit great variation in size and shape. Vesicle walls are two-layered, and the smooth, thick inner wall is surrounded by a thin, rough outer wall. Although highly vacuolate when they first appear, vesicles become increasingly dense and laden with oil globules as they age.

Vesicles are thought to function as both food storage organs and infectious chlamydospores (Butler, 1939; Nicolson, 1959). A recent ultrastructural study suggests that they function in either capacity (Kinden and Brown, 1975). Such vesicles are the only type of spore known for mycorrhizae in grass roots (Gerdemann and Trappe, 1973; Nicolson, 1959). External vesicles with attached hyphae may appear singly or in aggregates.

Although the literature does not mention at what stage in the host's development external vesicles are formed, internal vesicles are known to increase in number with the onset of flowering or fruiting (Saif, 1977). Formation of interior vesicles is considered to be a "constant" or mature state of endophyte development because the numbers of penetration hyphae become greatly reduced with their appearance. In grasses, interior vesicles can be released into the soil as spores after roots become senescent and the cortex is sloughed off. In some cases, the formation of interior vesicles is so profuse that the root becomes disrupted (Gerdemann and Trappe, 1973). Whether such disruptions are normal is questionable. Of additional interest is the finding that vesicles alone were produced on low productivity soils supporting cotton, while vesicle, arbuscule, and spore production all took place on similar high productivity soils (Bird *et al.*, 1974).

Both cytological and physiological changes induced in the host by the presence of mycorrhizae have been described. Gross morphological changes induced in the root system, such as ECM-induced differentiation into short infected and long roots in *Pinus* spp., are not obvious in grass roots. Baylis (1972a, 1974) has discussed the possible adaptive strategies of various root systems in relation to EDM infection. He has concluded that fatter roots with a persistent renewable cortex are much more dependent on mycorrhizae than those with highly reticulate, proteoid, or hairy roots or those with exceptionally abundant and long root hairs.

One can hypothesize the following: If a plant cannot maintain the mycorrhizal relationship or no longer needs the increased absorptive capacities for water and minerals which mycorrhizae provide, obvious environmental-physiological changes must have taken place to offset the loss of the endophyte. In the root system of a plant, such changes either denote that the plant has found another means to make up for the loss of the endophyte through possible variations in root structure or growth rate, or the plant no longer has to or is capable of competing with other community associates for nutrients, that is, that the community has become stabilized or is faced with drastic successional decline.

The purpose of the mycorrhizae investigation was first to ascertain the presence or absence of endomycorrhizae with *Agropyron smithii* Rydb. on the ZAPS sites. The very low SO_2 dosages needed to disrupt the symbiotic state, as exhibited by lichens and the known sensitivity of many fungi to sulfur have been discussed. In light of this sensitivity, it was further hypothesized that the symbiotic mycorrhizal state would also be disrupted at very low SO_2 dosage levels and that a downward trend in mycorrhizal populations would therefore be evident across the ZAPS plots. Conversely, various fumigation studies have reported that SO_2 stimulates photosynthesis and plant yield. It was thus conceivable that low SO_2 dosages might also have an initial invigorating effect on mycorrhizal populations.

MATERIALS AND METHODS

Sulfur Accumulation in Plants

Collection, sample preparation, and chemical analyses methods employed in 1977 were similar to those used in 1976 (Third Interim Report -- hereafter TIR), with the following modifications in collection procedures:

(1) An exclosure 2 km northeast of ZAPS I was chosen as a control plot (Off Plot Control or OPC) to avoid the effects of any fugitive SO_2. An area within the exclosure, with a vegetation community similar to that on the ZAPS sites, was divided into ten marked subplots. Some control sampling had been done in 1976 about 1.5 km southwest of ZAPS I, but this area was not enclosed, and sample size and frequency of sampling were limited.

(2) Subplot samples were kept separate unless the sample volume was so small as to necessitate a composite of vegetation from a particular treatment plot. This generally resulted in ten samples per tissue per species per treatment plot and allowed more detailed analysis of variability both within a treatment plot (between subplots) and between treatment plots (A, B, C, D, OPC).

(3) Several 1976 collections were separated by subplot. Plant tissues from perimeter subplots had lower sulfur levels than interior samples. Given our limited sampling resources, we decided to eliminate the suspected edge effect on data variability by sampling only interior subplots. This reduced the number of possible subplots per treatment from 86 to 44. For the division of a ZAPS plot into subplots, refer to Figure 14.41, which is presented later in this section.

(4) *Tragopogon dubius* was collected only once in 1976 (TIR). This species exhibited unusually high sulfur levels in contrast to the other species being studied. Because of this, *T. dubius* was sampled on a regular basis during 1977.

Data analyses of sulfur variates are facilitated by $\log_{10}$ transformations. These transformations help normalize the data and improve homogeneity of variances, allowing comparison of treatment plots by t and F tests. Screening data for statistical outliers (Grubbs, 1969; Dixon and Massey, 1957) also improved homoscedasticity. An observation was rejected from plot comparison only if it met all of the following criteria: (1) Preliminary screening for equal variance (Bartlett's chi-square) revealed heteroscedasticity; (2) the suspected value exceeded that suggested by the Dixon Criteria at the 95% significance level; (3) repeated chemical analyses established the veracity of the first chemical determination, and (4) the subplot exhibiting the unusual datum point demonstrated the same statistical aberration during at least one other collection period. The fourth criterion insured that the abnormality was not a sampling error but did, in fact, exist on the treatment plots. An example of the effects of removing outlying sulfur observations on mean values and 95% confidence intervals for total sulfur is presented in Appendix 14.1.

Linear regression proved useful in analyzing the sulfur accumulation data for the period after the spring greenup and through the growing season (TIR). The sulfur accumulation curves over the entire period of investigation suggest a polynomial of at least three degrees when there is no fall greenup (1976) and minimally four degrees when a fall greenup is observed (1977). To ignore quadratic and higher order components in SO_2 impact models will lead only to a gross oversimplification of the system and distort the accuracy of predictions. To date (March, 1978), we have made no attempt to ascertain the adequacy of our data base to describe the curvilinear relationships. We hope to do this in the future and thereafter make any necessary modifications in our collection procedures if sampling opportunities permit. Accordingly, no detailed analysis through time is presented herein, but the pattern of sulfur accumulation is portrayed as a review of graphs.

Washing Experiments

Agropyron smithii and *Koeleria cristata* samples collected in mid-September, 1976, on ZAPS IID were randomly split to determine both the sulfur content of plant material after a wash, simulating a heavy rainfall event and the sulfur leached from the plant material due to overwintering. The unprocessed plant parts were placed in a screened jar and rinsed with rapidly flowing tap water for three minutes to produce the effects of an intense individual rainstorm. Overwintering was simulated by washing the plant in distilled water for four days at room temperature. The water in the soaking experiment was changed after the second day. After washing or soaking, the material was dried and ground as usual for sulfur analysis. The data were examined with a paired-t test.

Fluoride Uptake

Live and dead tissue of both *A. smithii* and *K. cristata* collected from ZAPS IIA and IID in September, 1976, were analyzed for fluoride content. Methods were detailed in the TIR. The variance ratios were examined by the F test, and the equality of the mean fluoride levels was compared by an approximate F test based on synthetic degrees of freedom (Snedecor, 1956).

The 1977 vegetative phenology study was similar to our 1976 study (TIR), with the following modifications: (1) To reduce edge effects, the perimeter subplots were not used; (2) the number of subplots with tagged plants was increased from 10 to 20, thus expanding the number of marked plants per treatment plot from 50 to 100, and (3) the Off Plot Control (OPC) was included in the study. There were nine observation periods from early May through late September. *A. smithii* continued to be the only species scored. Tagged plants were located at a minimum distance from the delivery system pipes of one meter and a minimum of two meters from the nearest gas release point.

Seed Work

For the purpose of this study, germination is defined as the ability of the seed to produce two millimeters of radicle or epicotyl elongation under growth chamber conditions considered to be optimal for the species. This is to be distinguished from viability, which is the existence of a seed embryo in which all structures essential for the establishment of a seedling are alive and intact. Viability is determined by treating the seed with a vital stain (2, 3, 5-triphenyl tetrazolium chloride) to color the respiring tissues of the embryo. If the hypocotyl tip of a tap-rooted species does not stain, the embryo is not considered viable. However, a species capable of developing seminal roots can still establish a seedling even if the hypocotyl tip is dead. Viability then is an indication of the potential for successful seedling germination and does not include consideration of conditions for breaking dormancy or a favorable germination environment.

Seeds of four grasses (*Agropyron smithii, Koeleria cristata, Poa sandbergii,* and *Stipa viridula*) and a composite forb (*Tragopogon dubius*) were collected from both ZAPS sites during 1976. *A. smithii* and *T. dubius* were also collected from the Off Plot Control. Seed heads were clipped from a minimum of ten plants per treatment plot. The seed heads from each treatment plot were combined to form a single sample. The material was stored for approximately one year to allow after-ripening. Seeds were then hand-stripped from the heads.

Replicates for germination tests were obtained on a weight basis independent of the number of seeds. This technique serves to reduce sorting bias in obtaining subsamples and standardizes the total tissue mass in the trial. When the total weight of seeds from any treatment plot was limited, the available material was equally apportioned among four replicates. When larger seed volumes were available, enough seed to obtain a standard weight was randomly selected from the sample. In both cases, the total number of seeds per replicate was determined after weighing out the sample. The data on average seed weights were also derived from these subsamples. Replicate subsamples for tetrazolium viability tests were composed of fixed numbers of seeds. These seeds were removed from a grid table at randomly pre-selected intersections. Five replicates per treatment plot were used in the tetrazolium tests.

Germination tests were to proceed for 30- and 45-day periods, depending on species. These tests were conducted in growth chambers under moisture and temperature regimes which encourage bacterial and fungal growth. Two methods of restricting this fungal growth were pre-tested. Seeds were twice washed with a solution of Clorox and distilled water plus a wetting agent (12 cc Clorox, 500 cc distilled water, 3 drops Tween 80). A second method (Eddleman, 1977a) consisted of dry dusting the seeds with 50% Ortho Captan (N-[trichlormethyll]thio 4-Cyclohexene-1,2,-dicarboximide) and using an Ortho solution (two teaspoons per liter water) as the water supply for germination. In both cases, the petri dishes and cellulose pads which supported the seeds were autoclaved for 15 minutes at 15 pounds pressure. The Ortho treatment proved to be more effective in reducing fungal growth.

Seed storage requirements, stratification, and optimal germination conditions tend to be species specific. The work of Eddleman (1977a, 1977b) and Eddleman and Doescher (1977) provided the basis for selection of conditions and techniques suitable for germination testing. The procedures were modified for our particular study objectives. Basic methodologies for tetrazolium testing were derived from the Association of Official Seed Analysts (1970). Specific staining procedures for the species of interest were derived by pretrial experimentation.

After Ortho dusting, all seeds in a replicate weight were transferred to the sterilized cellulose pads and petri dishes under a laminar flow hood. Replicates were randomly assigned to a position in the growth chamber, then rotated daily after the germination counts were made. The number of seeds germinating each day was recorded, and at the end of the test period the number of ungerminated seeds was counted.

Basic data consisted of the number of seeds germinating on a particular day. The total number germinating during the test period was divided by the total number of seeds per replicate. Their percentages were subjected to arcsine transformation and treated by anova and Duncan's New Multiple Range Test (Duncan, 1955). Results are presented as untransformed percentages. Seed weights were analyzed by anova and Duncan's NMRT. Viability results were treated similarly to the percent germination. The rate of germination was analyzed as relative cumulative frequencies. The number of seeds germinating on any day was divided by the total number of seeds germinating and the result added to that from all previous days. The resultant relative cumulative frequency distributions were then compared by the Smirnov Test (Smirnov, 1939). The appropriate .95 quantiles were computed based on the asymptotic distribution for unequal n's:

$$W_{.95} \simeq \frac{m + n}{mn}$$

as suggested by Conover (1971). The rate of germination was integrated to a single number, the coefficient of rate of germination (CRG):

$$\sum_{1}^{n} \frac{g_n - g_{(n-1)}}{n}$$

where g_n is the accumulated germination on a given day minus the germination percent on the previous day ($g_{(n-1)}$) and divided by the number of days of incubation (n) (Maguire, 1962). The more rapid the "germination," the greater the CRG. This was modified in that g and $g_{(n-1)}$ were based on only those seeds which did germinate. Thus, assessment of germination speed was done independently of total germination success, as was the relative cumulative frequency distribution analyses. Days to 50% of final germination were also computed to compare germination speed of ZAPS seeds to those reported by Eddleman (1977b) as typical of southeastern Montana seed sources. Finally, the relative frequency distributions based on percent of total germination were graphed to display the day of peak germination and the pattern of germination timing.

Mycorrhizal Studies

In the spring of 1977, a paper entitled "Colorimetric Quantification of Vesicular-Arbuscular Mycorrhizal Infection in Onion," by Becker and Gerdemann, was published. The article presented an abbreviated method for quantifying EDM by using a spectrophotometer to measure the yellow coloration imparted to infected onion roots.

Spectrophotometer absorbance readings of the pigment, extracted from the roots with hot water were found to be highly correlated at 400 nm with percentage of yellow roots by weight. Percentage of yellow roots by weight also correlated well with length or, similarly, percentage of infected roots. Becker and Gerdemann suggested that the extraction method could be substituted for determination of percent infection by clearing and staining.

While uninfected onion roots are typically white, the exact nature of the yellow pigment, other than its tendency to break down with exposure to light, remains unknown. Our investigation included an attempt to determine whether such a pigment occurs in infected roots of *Agropyron smithii* and whether Becker and Gerdemann's method could be employed on vegetation from the ZAPS sites.

In the field, roots of *A. smithii* were not noticeably yellow, especially when compared to those of *Koeleria cristata*, although some appeared to be much more white or brown than others. *K. cristata* roots were found to be mycorrhizal, but *A. smithii* roots were used for this investigation because a larger percentage of the young active roots of this species were thought to be near the soil surface due to its rhizomatous habit.

Collections of whole plants from the ZAPS sites were made twice during the summer of 1977. To assure an adequate sample size, at least .5 g fresh weight of *A. smithii* were collected from each of the 90 ZAPS and OPC plots. Each of these root samples were separated from plants whose leaves and remaining roots were later analyzed for total sulfur content. In the laboratory, the roots were washed to remove soil, debris, and foreign and noticeably dead or decaying roots. Roots were blotted dry and weighed into .5 g samples immediately after washing. Samples were then processed in an autoclave at 121°C for 30 minutes to obtain a hot water extract.

After absorbance readings of root samples were completed, root infection was once again assessed by a modification of Newman's point intersection method as done by Sparling and Tinker (1975) and to some extent by Nicolson (1960). Such a method consists of placing the same .5 g root sample, now cleared by KOH and stained with trypan blue in lactophenol, in a petri dish at ten randomly chosen fields of vision under a binocular scope. At each field of vision, the ratio of mycorrhizal to non-mycorrhizal roots intersected by a rotated ocular graticule is then determined.

Data obtained from the determination of percentage of infected roots exhibited a considerable amount of variation within individual ZAPS plots. In most cases, assumptions of analysis of variance could not be met, and non-parametric methods were utilized instead. Whether a difference in infection levels existed between the plots was determined by the Kruskal-Wallis test. Further pairwise comparisons between each of the treatment plots were made with the Wilcoxon two-sample test.

RESULTS

Sulfur Accumulation in Plants

The pattern of sulfur accumulation from April through October, 1976, is portrayed in Figures 14.1 through 14.20 for *Agropyron smithii*, *Koeleria cristata*, *Achillea millefolium*, *Artemisia frigida*, *Stipa viridula*, *and Aristida longiseta*. The plotted values represent arithmetic means, and the data are tabulated in Appendix 14.2.

The spring greenup exhibited an initial peak in sulfur content. This was particularly evident on the A and B plots where accumulated sulfur from subsequent fumigation at the lower ambient concentrations did not mask the greenup. Nutrient levels were relatively high compared to biomass in spring grass. Rapid increase of aboveground tissue weight then led to relatively less sulfur. Under continued fumigation, sulfur levels increased until mid-July and then began to decline as growth ceased. Although fall greenups are normal in prairie regions when moisture is adequate, this did not occur in 1976.

Sulfur introduced into ecosystems may continue to cycle in that system either by storage in plant tissues or by availability in the soil system. The buildup of biologically available sulfur may be small from any one year to the next. However, if input is intense, or of low level but long duration, a detrimental increase could be expected to occur. Sulfur initially introduced by fumigation and carried over into live tissue in subsequent years will be defined as residual sulfur.

The first collection of live plant material in 1976 on ZAPS I was made after one year of fumigation (1975) but before the 1976 fumigation began. One might expect to see the greenup sulfur levels to be the highest on ZAPS ID and lower on IA. This "residual sulfur" trend was exhibited by *A. smithii*, *A. millefolium*, and *A. frigida* but was reversed in *K. cristata*.

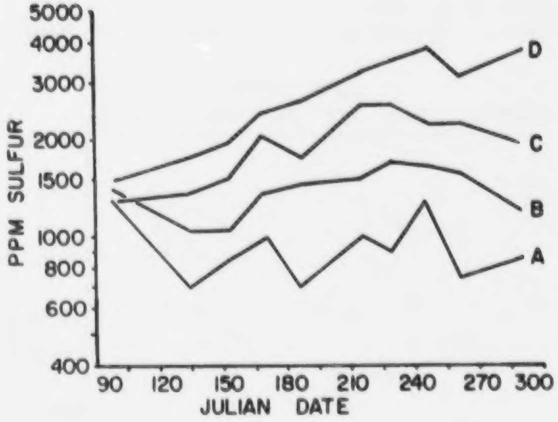


Figure 14.1. Average 1976 sulfur levels in live *A. smithii* from ZAPS I.

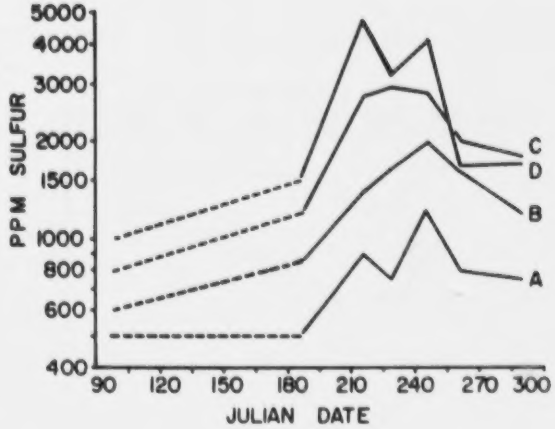


Figure 14.2. Average 1976 sulfur levels in dead *A. smithii* from ZAPS I.

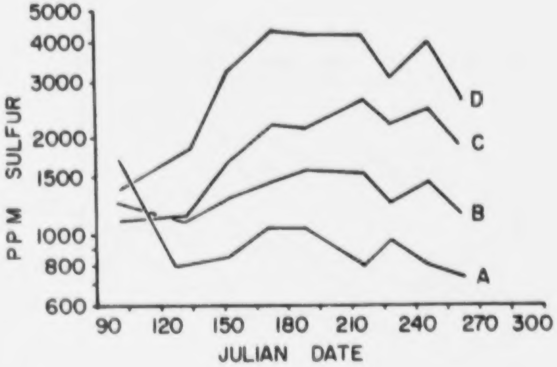


Figure 14.3. Average 1976 sulfur levels in live *A. smithii* from ZAPS II.

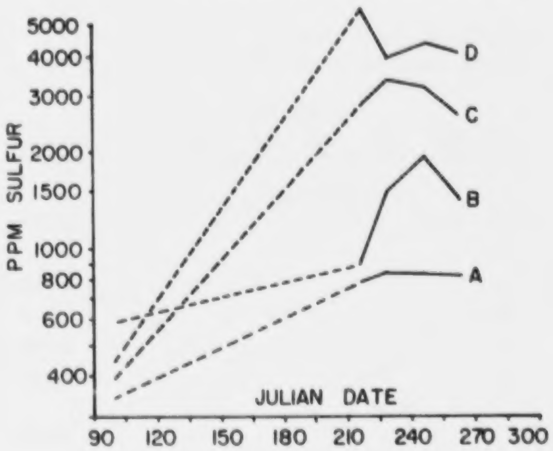


Figure 14.4. Average 1976 sulfur levels in dead *A. smithii* from ZAPS II.

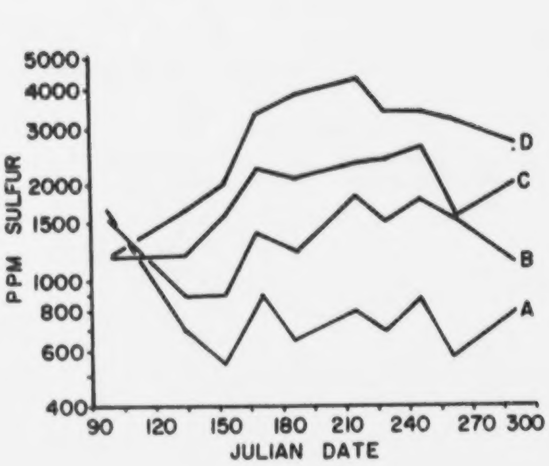


Figure 14.5. Average 1976 sulfur levels in live *K. cristata* from ZAPS I.

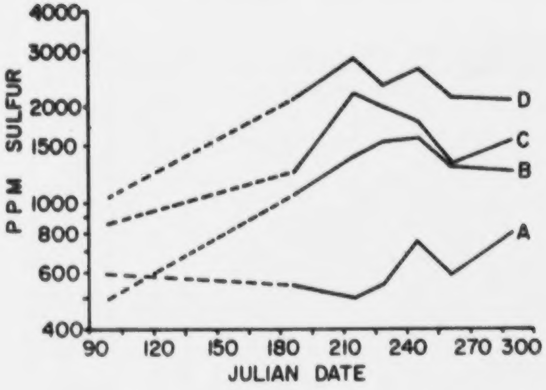


Figure 14.6. Average 1976 sulfur levels in dead *K. cristata* from ZAPS I.

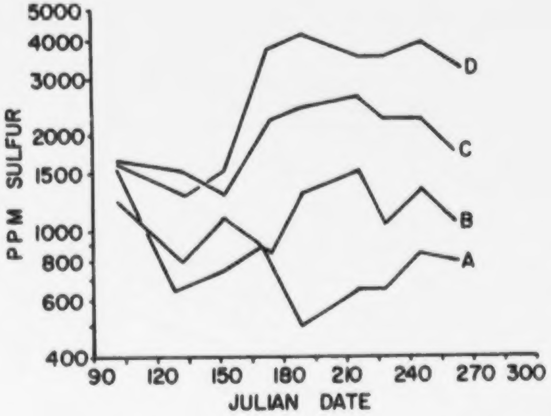


Figure 14.7 Average 1976 sulfur levels in live *K. cristata* from ZAPS II.

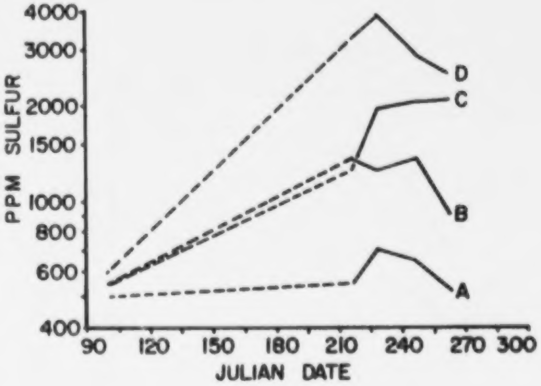


Figure 14.8. Average 1976 sulfur levels in dead *K. cristata* from ZAPS II.

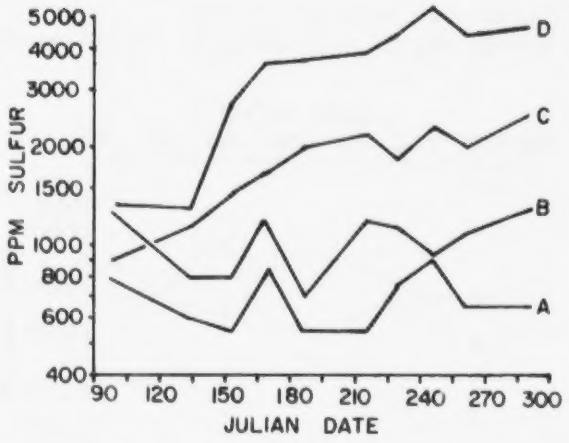


Figure 14.9. Average 1976 sulfur levels in live *A. millefolium* from ZAPS I.

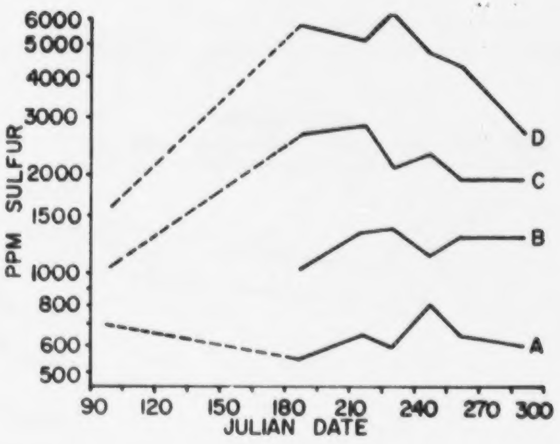


Figure 14.10. Average 1976 sulfur levels in dead *A. millefolium* from ZAPS I.

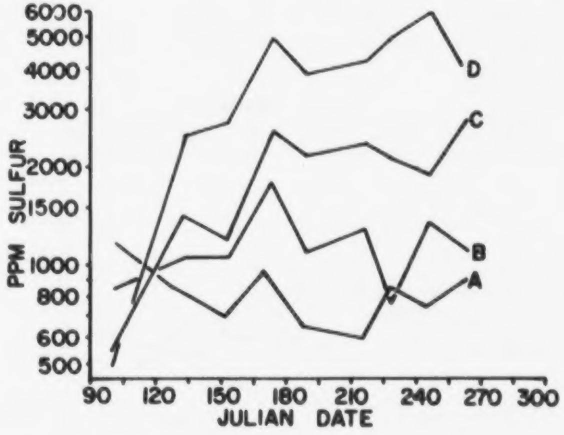


Figure 14.11. Average 1976 sulfur levels in live *A. millefolium* from ZAPS II.

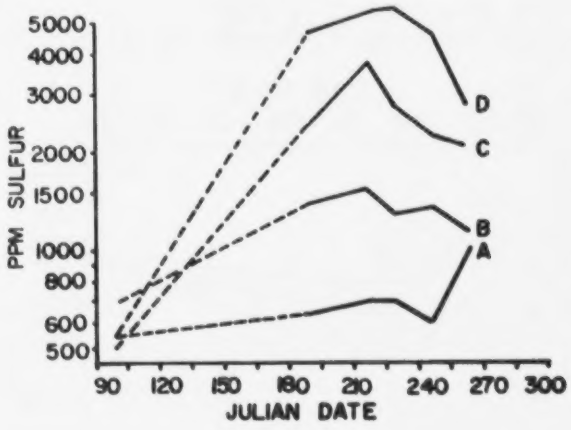


Figure 14.12. Average 1976 sulfur levels in dead *A. millefolium* from ZAPS II.

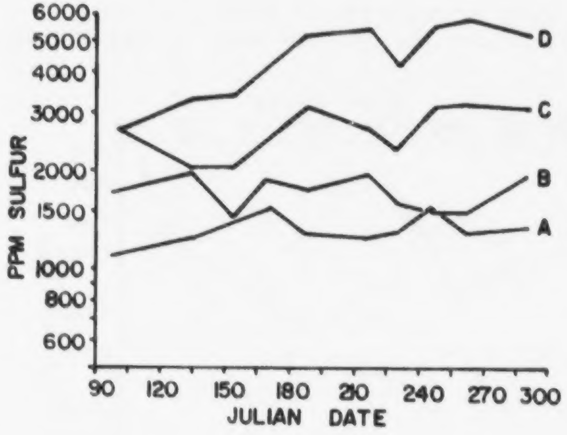


Figure 14.13. Average 1976 sulfur levels in live *A. frigida* from ZAPS I.

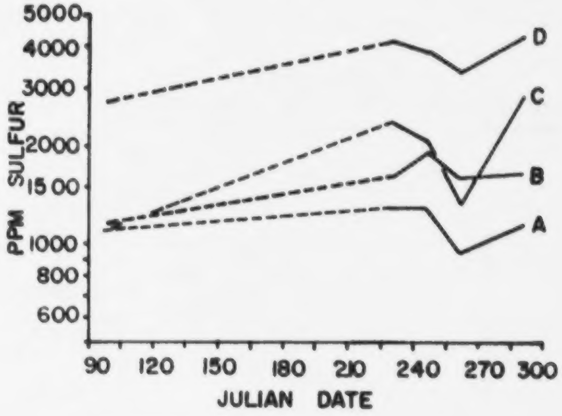


Figure 14.14. Average 1976 sulfur levels in dead *A. frigida* from ZAPS I.

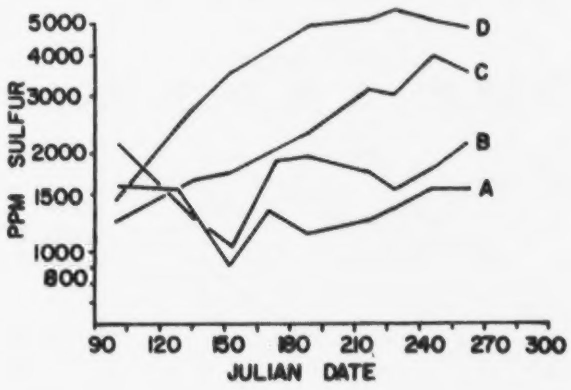


Figure 14.15. Average 1976 sulfur levels in live *A. frigida* from ZAPS II.

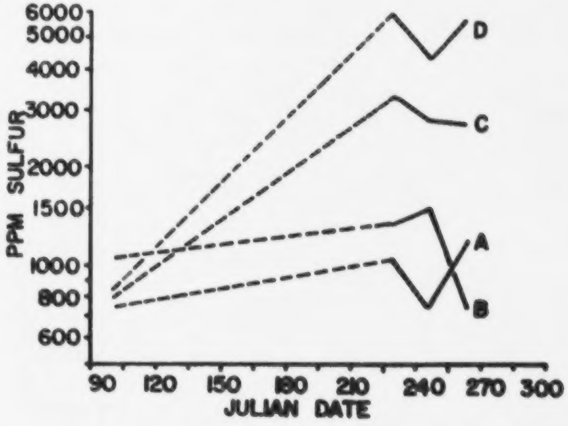


Figure 14.16. Average 1976 sulfur levels in dead *A. frigida* from ZAPS II.

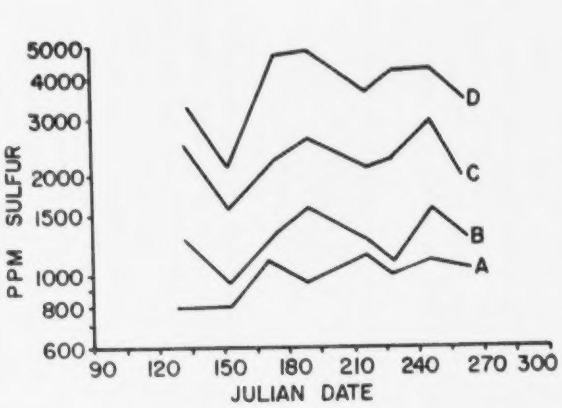


Figure 14.17. Average 1976 sulfur levels in live *S. viridula* from ZAPS II.

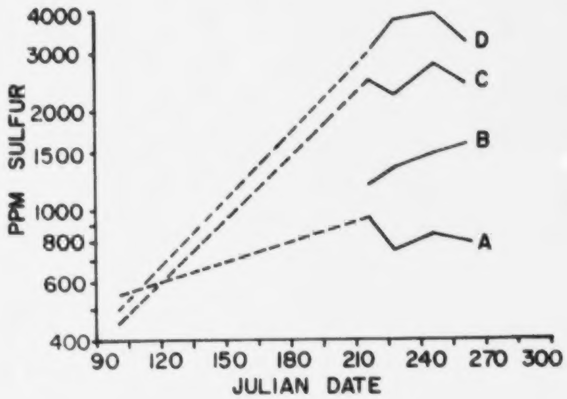


Figure 14.18. Average 1976 sulfur levels in dead *S. viridula* from ZAPS II.

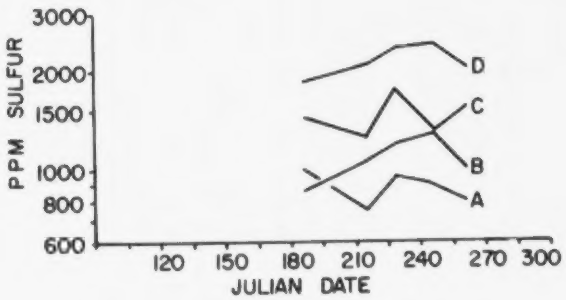


Figure 14.19. Average 1976 sulfur levels in live *A. longiseta* from ZAPS I.

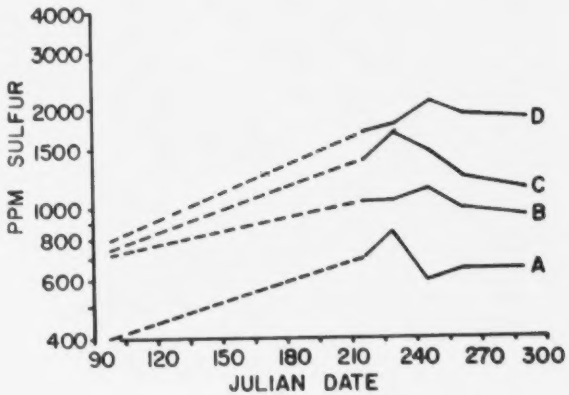


Figure 14.20. Average 1976 sulfur levels in dead *A. longiseta* from ZAPS I.

Sulfur determination at the initiation of fumigation in 1977 provides some additional data (Figures 14.21 and 14.22). ZAPS II now has been fumigated for two years. *A. smithii* exhibits residual sulfur on both ZAPS I and II (chemical analyses of other species have not been completed). These relative levels are summarized as ranks in Table 14.1 (1 being the lowest sulfur content; 4 the highest) for all cases applicable to testing the hypothesis. An analysis by Friedman's two-way anova yields: P = .136. *K. cristata* data runs counter to the trend and thus reduces the χ^2 (chi-square) value.

TABLE 14.1. RESIDUAL SULFUR BUILDUP (FRIEDMAN'S TEST)

			Plot			
Species	Site	Year	A	B	C	D
A. smithii	I	1976	1.5	2	1.5	4
A. smithii	I	1977	1	3	2	4
A. smithii	II	1977	1	2	3	4
K. cristata	I	1976	4	3	1	2
A. millefolium	I	1976	1	3	2	4
A. frigida	I	1976	1	2	3	4
		ΣR_i =	9.5	15	12.5	22

$\chi^2_r = 5.55$ df = 3 P = .136

The collection of multiple subplot samples in 1977 allowed 95% confidence limits to be set for the various collections. Examination of collection 21 (made at the initiation of fumigation) reveals that samples from all ZAPS plots had significantly higher sulfur levels ($\alpha = .05$) than samples from the OPC and that vegetation from both ID and IID displayed significantly higher concentrations than samples from the other plots (Figure 14.27). These data support the concept of residual sulfur, at least in the case of *A. smithii*.

The recently dead tissue of both *A. smithii* and *A. millefolium* contained more sulfur than the live on B, C, and D plots (Figures 14.2, 14.4, 14.10, and 14.12). Dead tissues of *K. cristata*, *A. frigida*, *A. longiseta*, and *S. viridula* generally contained less sulfur than the live tissues.

The 1977 collection will allow statistical analyses of those observations. The partitioning of sulfur (or any element) in live and dead tissues is of interest in the following contexts: (1) Leaf senescence often is preceeded by translocation of nutrients to other plant tissues; (2) compartmentalization of toxins, particularly in tissues about to be shed, is a possible detoxification strategy; (3) older tissues can be expected both to have received higher dosages and to senesce first, and (4) sulfur deposited in "dead" tissues becomes an input source to other compartments (decomposers, grazers, soil) through biological breakdown and physical leaching (see Washington Experiments).

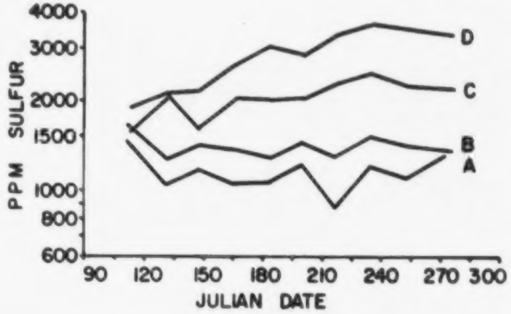


Figure 14.21. Average 1977 sulfur levels in live *A. smithii* from ZAPS I.

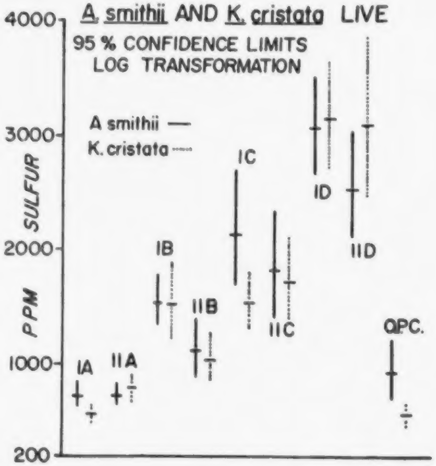


Figure 14.23. Sulfur levels in live *A. smithii* and *K. cristata* from both ZAPS in 1976.

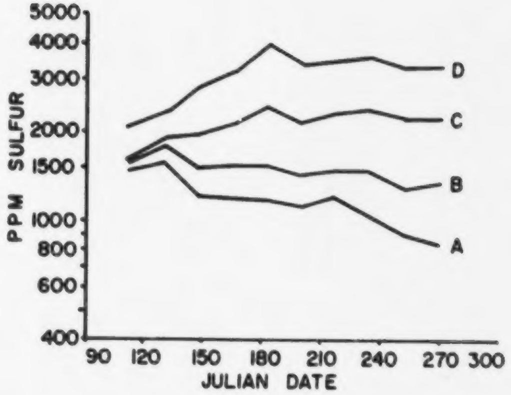


Figure 14.22. Average 1977 sulfur levels in live *A. smithii* from ZAPS II.

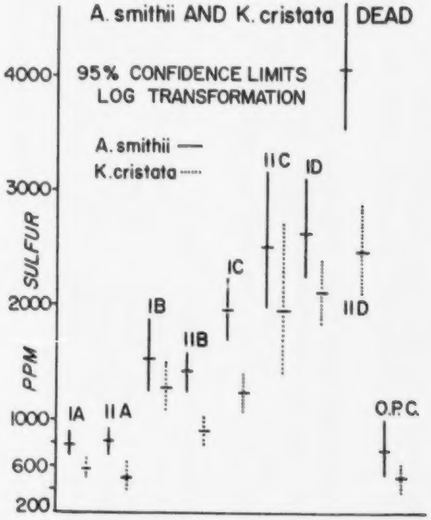


Figure 14.24. Sulfur levels in dead *A. smithii* and *K. cristata* from both ZAPS in 1976.

The leaves of live *A. smithii* and *K. cristata* seemed to contain similar amounts of sulfur (Figure 14.23) on six of the nine plots in mid-September, 1976. *K. cristata* contained less sulfur on the OPC, IA, and IC plots. An examination of sulfur levels throughout the 1976 season suggests that on the B, C, and D plots (Figures 14.1 and 14.5; Figures 14.3 and 14.7), the two species had similar sulfur content and that the depression of *K. cristata* on IC was due to sampling variability. *K. cristata* from both A plots had less sulfur during most of the year than *A. smithii*.

Although the live tissues of *K. cristata* and *A. smithii* exhibited similar sulfur levels, the dead leaves of *K. cristata* contained less sulfur than those of *A. smithii* (Figure 14.24). It is also of interest to note that the sulfur content of dead *A. smithii* on IID was greatly elevated.

Across treatment plots (A, B, C, D) and within sites (I, II), the sulfur accumulation tended to differ significantly (Table 14.2), but this was not invariably the case. Across-site comparisons of purportedly similar or dissimilar treatments do not always meet expectations of sulfur accumulated. As an example, compare live tissue of IB and IIB or IB and IIC (Figure 14.23). This suggests that the a priori combining of other treatment results from the two sites may not always be desirable, as this assumes equal dosages received by the plant canopy.

TABLE 14.2. SULFUR ACCUMULATION IN MID-SEPTEMBER, 1976 (COLLECTION 9)

Species	Material	Site	Plot A	Plot B	Plot C	Plot D
A. smithii	Live	I	731	1538	2143	3059
A. smithii	Live	II	735	1122	1820	2547
A. smithii	Dead	I	490	1549	1961	2639
A. smithii	Dead	II	814	1429	2505	4068
A. smithii	Roots	I	728a*	733a	718a	827
A. smithii	Roots	II	719a	773ab	786b	866
K. cristata	Live	I	568	1524a	1552a	3150
K. cristata	Live	II	790	1044	1731	3101
K. cristata	Dead	I	581	1286a	1244a	2115
K. cristata	Dead	II	502	903	1956	2467
K. cristata	Roots	I	756ab	721a	828b	966
K. cristata	Roots	II	729a	772a	822a	927

*Means sharing the same letter do not differ significantly ($\alpha = .05$); Duncan's Multiple Range Test, Log_{10} transformation applied.

Belowground sulfur levels in both *K. cristata* and *A. smithii* increased with the intensity of fumigation (Table 14.2). The ratio of increase across the plots was much less than seen in tops during these first few years of fumigation. Higher sulfur levels in roots in the fall on higher fumigation plots are one possible source of the "residual sulfur" carried into the subsequent growing season.

Root sulfur levels tended to be similar for both species, but *K. cristata* were somewhat more variable. Also, on IC and ID, sulfur concentrations in *K. cristata* were significantly elevated relative to *A. smithii* (Figure 14.25).

The accumulation curves for live *A. smithii* in 1977 are presented in Figures 14.21 and 14.22. The spring peak is evident on the A and B plots but, again, masked by continuing fumigation on the C and D plots. The sulfur content of the grass from the C and D plots increased through the first half of the fumigation period but decreased or remained static on the A and B plots. This is an interesting contrast to the pattern of accumulation in 1976 on the B plots and reflects the limited soil moisture in the spring. The rate of change also decreased on the C and D plots in 1977, although the peak values were only slightly depressed. A major and consistent difference was found on the A plots where the levels were elevated over those observed in 1976. A fall greenup occurred in 1977, with a resultant relative increase in live tissue sulfur. This was evident on the OPC, IA, and IIA plots (Figure 14.26) when contrasted to the previous collection period (late October vs. late September). The means of samples from these plots exceeded 1,500 ppm, levels which were not seen except during the spring greenup.

The 95% confidence limits for sulfur levels in *A. smithii* throughout the 1977 fumigation period are portrayed in Figures 14.27 through 14.36. Data are tabulated in Appendix 14.3. Concentrations in vegetation from ZAPS II were often higher than in samples from corresponding treatments on ZAPS I through mid-July, but they were similar later in the season. Differences were usually significant across the plots.

Root sulfur levels in 1977 also tended to rise with increasing levels of fumigation treatment, but unanticipated shifts also were observed (Figures 14.37 through 14.39). The IIB *A. smithii* roots had elevated sulfur levels relative to samples from the other low and medium plots. Sulfur concentrations in the IC and ID mid-July collections seem particularly depressed relative to other plots.

The root sulfur levels in late April (collection 21), mid-July (collection 26), and mid-September (collection 29) are contrasted in Figure 14.40. The pattern appears to be one of decreasing sulfur levels, with the exception of samples from IC and ID which showed a sharper decline in mid-July. The smallest change was observed on the OPC. In spite of these treatment plot irregularities, significant correlation was observed between the sulfur content of live tops and their roots. In the mid-July collection, 88 samples from the nine treatment plots gave a Spearman's Rank Correlation of .301 ($P < .005$), and 41 matched samples from the OPC and ZAPS II had an associated Spearman's rho of .312 ($P < .025$).

The 1976 sulfur accumulation data suggested an edge effect in dosage delivered. Perimeter subplots, particularly those on the corners and extremities of the plots, such as 1, 5, 11, 21, and 25 (Figure 14.41), seemed consistently low for the treatment plot from which they were collected. This led to the modification of collection procedures to escape edge effects.

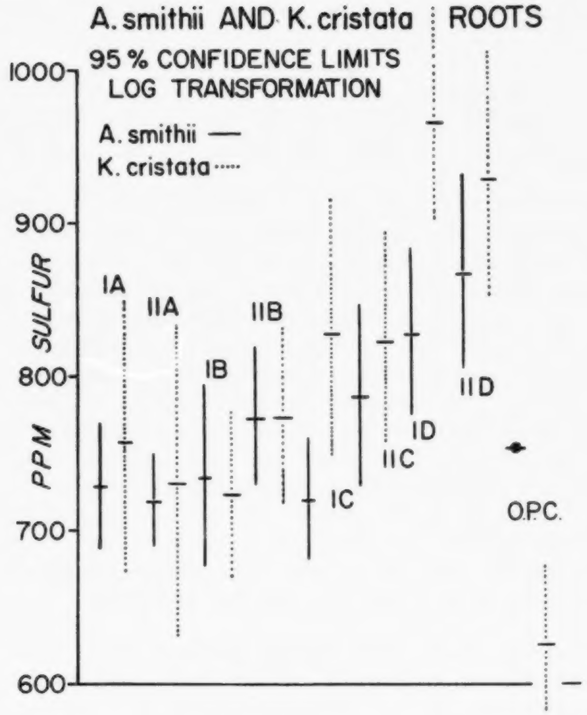


Figure 14.25. Root sulfur levels in *A. smithii* and *K. cristata* from both ZAPS during mid-September, 1976.

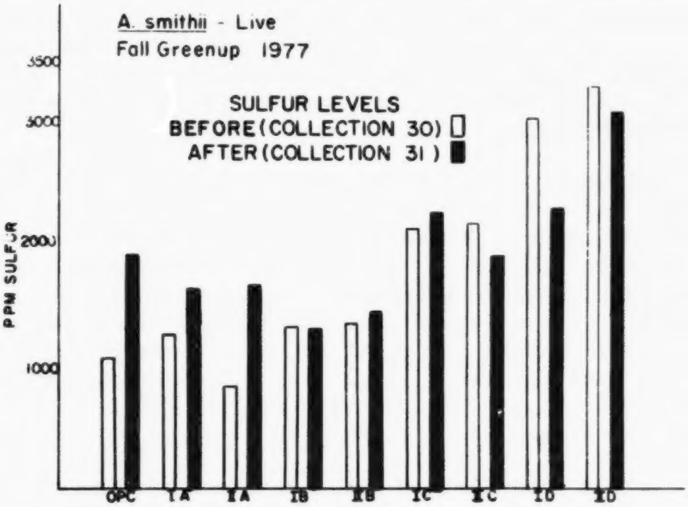


Figure 14.26. Sulfur levels in live *A. smithii* from both ZAPS during the fall greenup in 1977.

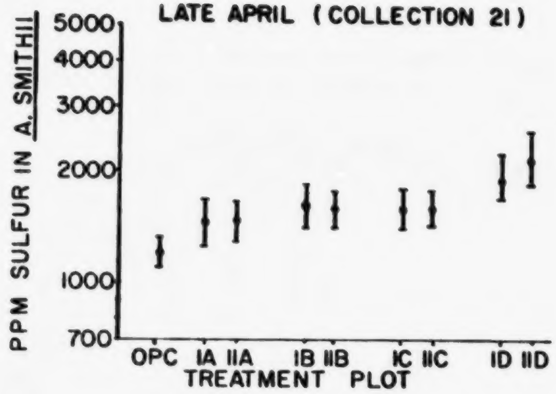


Figure 14.27. Sulfur levels in *A. smithii* during late April, 1977.

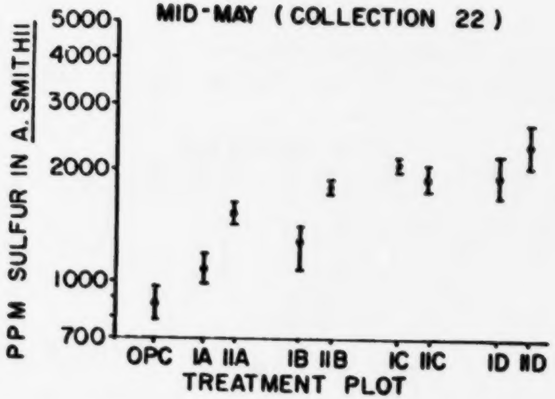


Figure 14.28. Sulfur levels in *A. smithii* during mid-May, 1977.

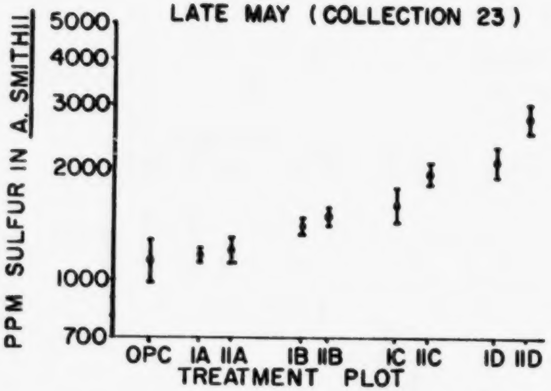


Figure 14.29. Sulfur levels in *A. smithii* during late May, 1977.

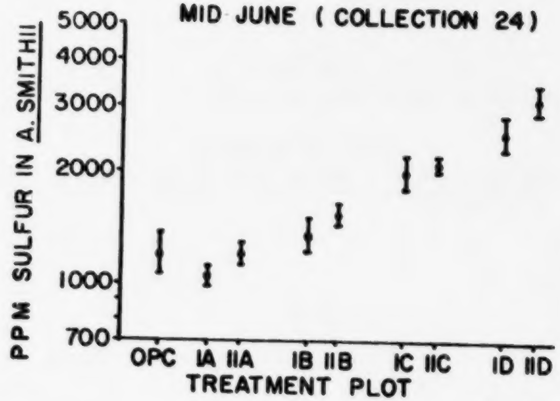


Figure 14.30. Sulfur levels in *A. smithii* during mid-June, 1977.

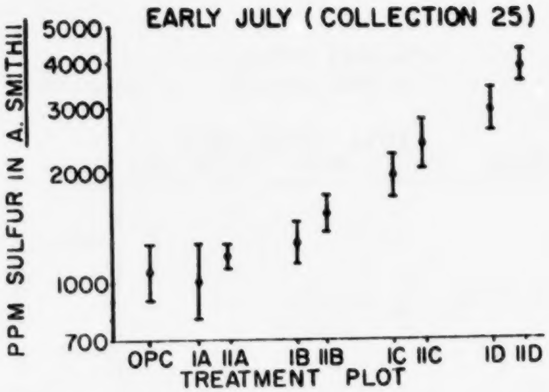


Figure 14.31. Sulfur levels in *A. smithii* during early July, 1977.

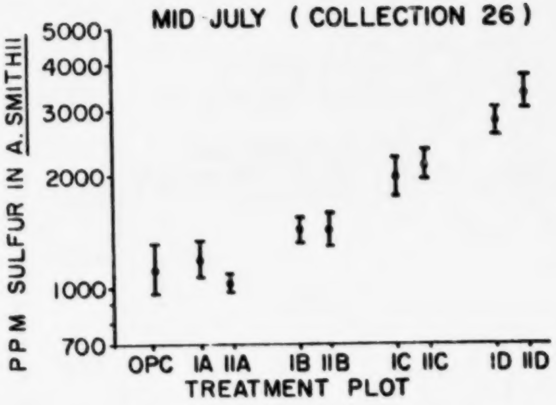


Figure 14.32. Sulfur levels in *A. smithii* during mid-July, 1977.

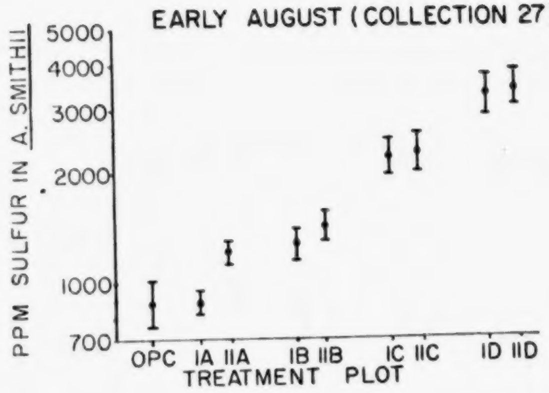


Figure 14.33. Sulfur levels in *A. smithii* during early August, 1977.

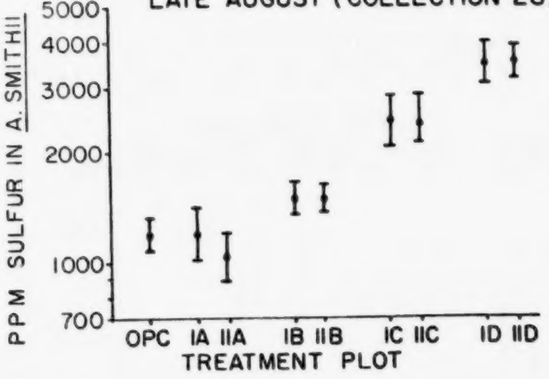


Figure 14.34. Sulfur levels in *A. smithii* during late August, 1977.

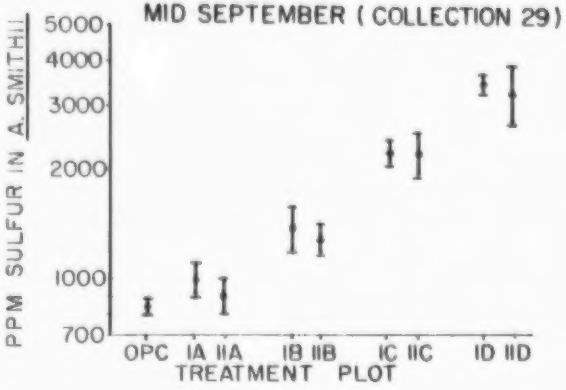


Figure 14.35. Sulfur levels in *A. smithii* during mid-September, 1977.

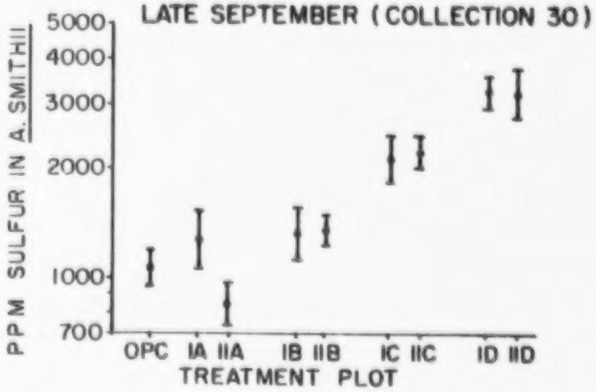


Figure 14.36. Sulfur levels in *A. smithii* during late September, 1977.

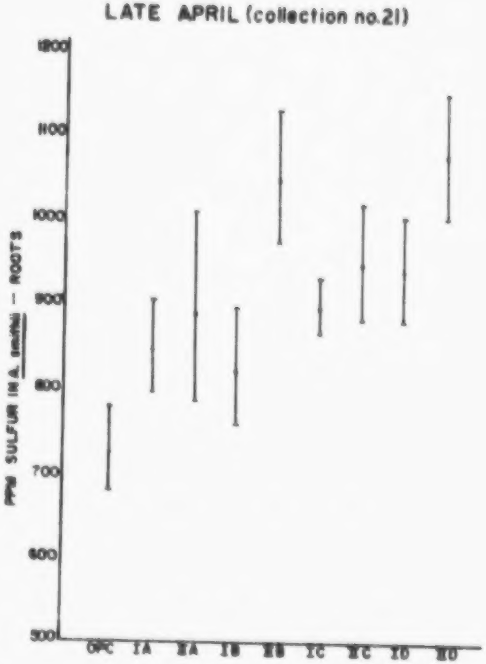


Figure 14.37. Root sulfur levels in *A. smithii* during late April, 1977.

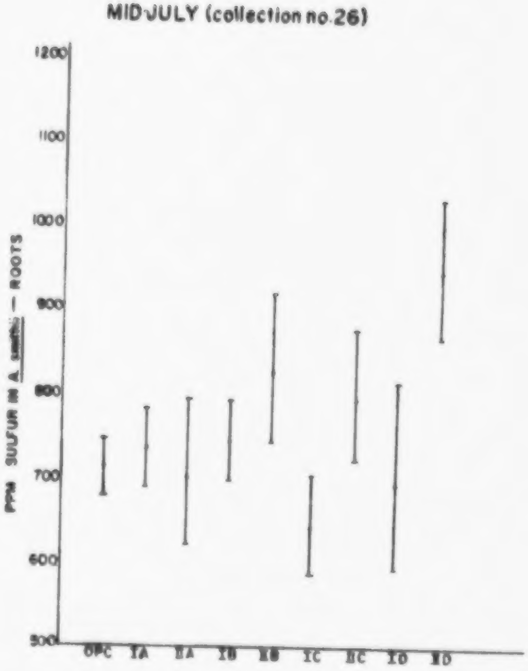


Figure 14.38. Root sulfur levels in *A. smithii* during mid-July, 1977.

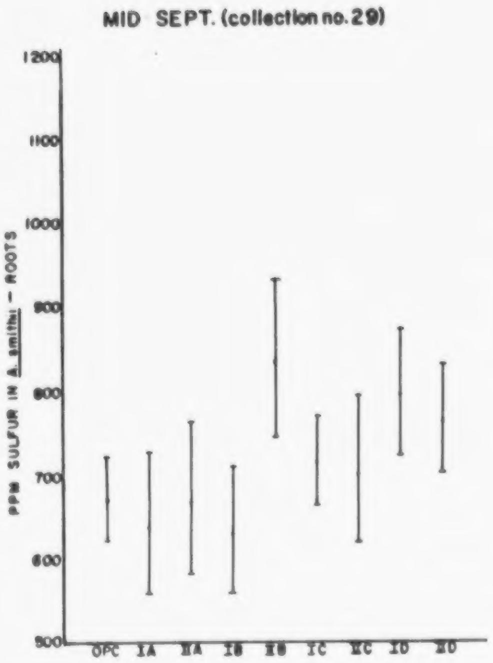


Figure 14.39. Root sulfur levels in *A. smithii* during mid-September, 1977.

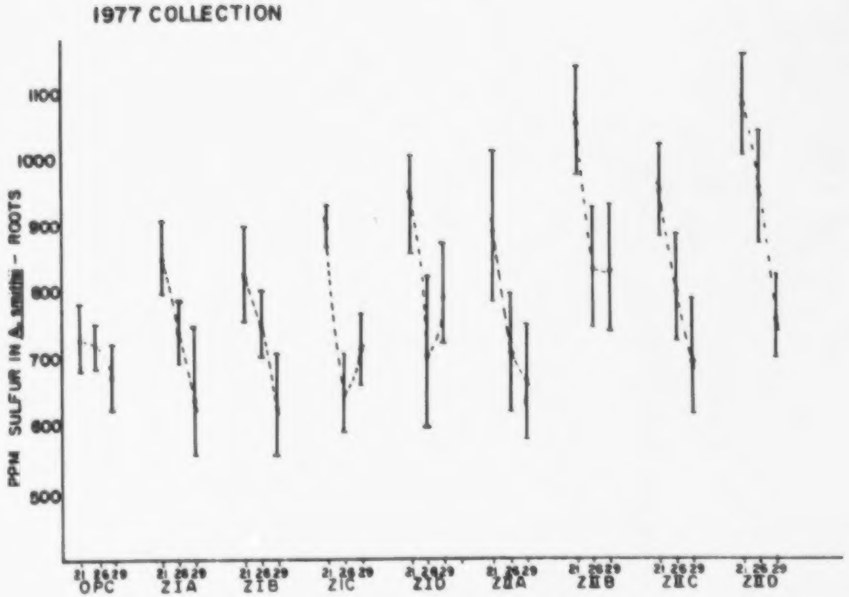


Figure 14.40. Root sulfur levels in *A. smithii* during late April (21), mid-July (26), and mid-September (29), 1977.

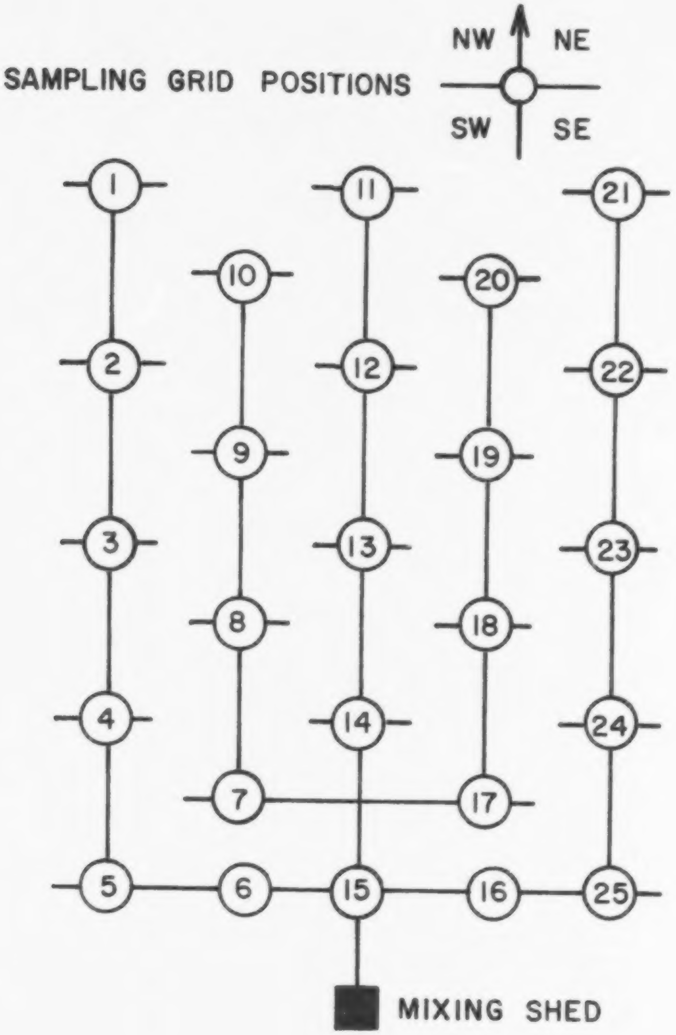

SAMPLING GRID POSITIONS
NW
NE
SW
SE
1
11
21
10
20
2
12
22
9
19
3
13
23
8
18
4
14
24
7
17
5
6
15
16
25
MIXING SHED

Figure 14.41. Subplot junction numbers and sampling grid positions.

The perimeter pipe lines were not sampled in 1977, and the same series of subplots (blocks) were sampled on all treatment plots for an individual collection period. Even after the elimination of the most obvious problem areas, significant subplot variability remained within treatment plots B, C, and D (Table 14.3). This difference in horizontal distribution of dosage was quite pronounced in some collections and less so in others (P exact ranged from .0032 to .6306). An overall analysis of combined probabilities (Table 14.3) (Fisher, 1954) indicated very highly significant differences within the treatment plots in terms of sulfur concentrations. Since similarly designated subplots on different treatment plots share in common only their spatial relationship to the gas delivery system, this difference in plant tissue sulfur is not due to edaphic features or inherent in the plants themselves.

TABLE 14.3. COMBINED PROBABILITIES FROM TWO-WAY ANOVAS TO DETECT WITHIN-TREATMENT PLOT DIFFERENCES IN DOSAGE DELIVERED

Collection Number	F_s (Subplots)	df_1, df_2	P	ln P
21	1.461	5,25	.2393	-1.4300
22	2.941	3,15	.0697	-2.6636
23	.786	9,45	.6306	-0.4611
24	1.464	8,40	.2011	-1.6040
25	1.412	7,35	.2327	-1.4580
26	3.231	9,45	.0043	-5.4491
27	3.529	8,40	.0061	-5.0995
28	.889	8,45	.5426	-0.6114
29	3.369	9,45	.0032	-5.7446
30	1.349	5,45	.2449	-1.4069
$k = 10$				$\Sigma = -25.9281$
$\nu = 2k = 20$	$\chi^2 = -2 \ln P = 51.856$			$P < .001$

The collections were examined in more detail to ascertain the pattern of dosage difference (Table 14.4). Only complete blocks were examined. The end of pipe and lower corner subplots (such as 10NW and 7SW) received the low dosages, while more central subplots (such as 9NW and 14SW) received the high dosages. This pattern is similar to that encountered in 1976 when all five pipelines were sampled.

Drift Plots

Aerial imagery of ZAPS I taken in 1975 was analyzed for SO_2-induced stress by Calspan Corporation (Schott *et al.*, 1976) and Montana State University (Taylor, 1976). Both groups delineated suspected SO_2-impacted areas surrounding the intentionally-fumigated plots. The "drift plots" (TIR) were established in 1976 in an attempt to provide ground level verification of the photographic techniques by measuring the relative sulfur content of

TABLE 14.4. ORDERED SUBPLOT DIFFERENCES IN DOSAGE FOR PLOTS B, C, AND D DURING 1977

Collection Number	Subplots*									
21	10SW	20NW	8SW	7SE	18SW	14SE				
22	17SW	12NE	18SW	20SW						
23	12SW	8SW	10NW	19SW	7NE	14SE	18NE	10SE	9NE	14NE
24	20SE	7SE	13NW	12SE	13NE	9SW	18SE	9NW		
25	7SW	17SW	8NW	10SE	14NW	17NE	14SW	18SW		
26	10NW	17NW	7NW	12SW	18SE	9SW	19SW	8NE	9NW	
27	7SE	7SW	20NE	20NW	12NE	19SE	13NW	9SE	14SW	
28	8NW	7SW	17NW	12SW	10SW	8SW	20SW	12NW	14SE	
29	10NE	8SW	17SE	9NE	20SE	8SE	19NW	14NW	12SE	19NE
30	10NW	7NE	10SE	19SE	8SW	17NW	13SW	14SW	12SE	12NE

*Underlined subsets do not differ significantly ($P \leq .05$) (Duncan's Multiple Range Test). Subplots are ordered from those receiving the lowest dosage to those receiving the highest dosage.

plant tissues in the suggested zones adjacent to the treatment plots. Using the mid-July, 1976, plant collection, we could not identify a cluster pattern of differing sulfur levels within the drift areas (TIR). The drift plots vegetation had significantly lower sulfur concentrations than vegetation from IB. However, the mean values for the drift plots tended to be higher than those for plot IA tissues but were never significantly so ($P = .05$).

The mid-September, 1976, drift plot collections are discussed in the following paragraphs. The ZAPS I treatment plots and drift plots all were collected within one day of each other in mid-September. The following comparisons will be made without prediction based on regression through time, as was necessary for the mid-July analyses (TIR).

On the drift plots as a whole, sulfur concentrations in the green leaf tissue of both *A. smithii* and *K. cristata* were higher ($\alpha = .05$) than those in both the necrotic leaf material and the belowground parts. *K. cristata* green leaf and belowground tissues had higher sulfur values than the respective

A. smithii material, while concentrations in tissue categorized as dead were similar for both species (Table 14.5). The correlation for sulfur in the green tissue was examined to determine whether there was any correspondence by specific drift plot between the two species. A positive correlation was assumed (one-tailed test, α = .05) and a significant Spearman's rho (.3065) was computed. This confirmed that the plants on certain drift plots were enriched as a function of location.

TABLE 14.5. SULFUR LEVELS IN MID-SEPTEMBER, 1976, DRIFT PLOT COLLECTIONS

Species Material	$\bar{x}$ ppm Sulfur	95% Confidence Limits (log transformations)	n
A. smithii			
Live	752	692-818	35
Dead	673	615-737	36
Roots	613	737-690	18
K. cristata			
Live	944	865-1029	39
Dead	702	639-772	40
Roots	693	642-749	20

Three approaches were used in an attempt to identify specific clusters of high readings. No approach was considered successful, but each will be described briefly.

(1) The 1975 photo interpretations (stress contours) were applied as an overlay to a grid derived from the 1976 sulfur readings. The photographically-identified areas of interest were large compared to the ground level sulfur grid (in excess of 50%), and/or the hierarchy of stress intensity provided limited resolution (three levels from Montana State University, six possible levels from Calspan). The IC and ID plots were easily identifiable, but the resolution of both photo maps and the ground level grid was inadequate.

(2) The second approach was a runs test above and below the median. The sulfur levels for particular tissue/species were categorized as being greater or less than the median and sequenced by the distance of the particular drift plot from the ZAPS sites. This necessitated the reduction of ZAPS I to a point source with emissions centered

approximately 25 m northwest of the western edge of the ID plot. The number of runs was always non-significant ($\alpha = .05$) but tended towards the low side, suggesting a contagious pattern. The major weakness in the approach was, of course, the reduction of the site to a point source with a hypothetical center of emissions. A ground level grid much more widely dispersed, relative to the ZAPS I, would be desirable.

(3) The third approach was an examination of sulfur levels of four tiers of drift plots (TIR, Table 13.2, p. 408) relative to the ZAPS plots. Concentrations in live *K. cristata* were found to be elevated in the two tiers closest to the treatment plots, relative to the two most distant tiers. Dead *K. cristata* did not repeat this pattern. Live and dead *A. smithii* tended to exhibit higher sulfur levels in tiers adjacent to the ZAPS sites, but this trend was not consistently significant.

In summary, analysis of sulfur levels in plant tissues did not confirm the photo imagery. This conclusion does not constitute a denial of the photographic interpretation. The design of the ground level grid was inadequate for confirmation. In order to confirm, or invalidate, the imagery using vegetation analyses, the following requirements would have to be met: (1) Interpreted imagery should be quickly returned to field personnel (minimally within the same growing season); (2) the scale of imagery maps and photos should be similar to that of the USGS 7½-minute series topographic maps or smaller; (3) the ground level sampling grid should cover a substantially larger area than that employed in 1976, and (4) the density of the ground level sampling grid should be adjusted to allow identification of change in areas where suspected SO_2 stress is rapidly changing.

Anaconda Sites

During mid-February, 1977, *A. smithii* was collected at several sites in the vicinity of the Anaconda copper smelter in Anaconda, Montana. Chemical analyses of these plant samples revealed sulfur concentrations far in excess of those expected, based on the ambient concentration at these sites, in comparison with our experimental work at the ZAPS sites. Three additional *A. smithii* collections were made in 1977 at two Anaconda sites where ambient SO_2 levels have been determined with continuous analyzers.

The Highway Junction site is 3 km northeast of the smelter stack and had an average ambient SO_2 concentration of 1.09 pphm for the period of active growth (April through July 15) and 1.96 pphm for the photosynthetic day (7 am to 4 pm). This is an arithmetic average, which is the customary manner of summarizing SO_2 monitoring data in the state of Montana. The data are from 1976, the most recent year for which tapes were available (Montana State Department of Health, Air Quality Bureau), but are quite typical of the concentrations historically recorded at that site. The Pumphouse site is located approximately 9 km northeast of the smelter. The average arithmetic SO_2 concentration of the April through mid-July, 1976, period was .43 pphm, a reduction corresponding to its increased distance from the smelter.

Climatic regimes at the Anaconda and the ZAPS sites are similar (Figure 14.42), although the Anaconda weather data, unlike Colstrip data, include the drought period of the 1930s.

Sulfur concentrations in live and dead *A. smithii* from the Highway Junction site (Table 14.6) equalled or exceeded the sulfur content of plants from the ZAPS D plots at equivalent times in the growing season. Belowground tissue levels were threefold to tenfold higher than those observed in samples from the D plots. Sulfur levels in the aboveground samples from the Pumphouse site equalled or exceeded those of the C plots. Roots from this site had concentrations three to seven times higher than roots from the C plots. This occurred at ambient concentrations more typical of the ZAPS A or B plots (Table 14.7).

TABLE 14.6. SULFUR CONCENTRATIONS (PPM) IN *AGROPYRON SMITHII* FROM ANACONDA SITES DURING 1977

	Pumphouse Site			Highway Junction Site		
	Late May	Mid-July	Mid-Sept	Late May	Mid-July	Mid-Sept
Live	2075	2009	2490	2696	3081	3935
Dead	1855	--	2450	5900	--	4551
Roots	3700	5595	2925	2642	4607	8882

TABLE 14.7. ARITHMETIC MEAN AMBIENT SO$_2$ CONCENTRATIONS (PPHM) AT ANACONDA SITES AND ZAPS A AND B PLOT PROBES FOR THE ACTIVE GROWTH PERIOD (APRIL THROUGH MID-JULY)

Site*	Probe‡	All Day	7 am to 4 pm
Anaconda	Highway Junction	1.09	1.97
	Pumphouse	.43	--
			6 am to 3 pm
ZAPS	IA$_c$	.90	.76
ZAPS	IIA$_c$	.78	.76
ZAPS	IB$_d$	4.79	4.22
ZAPS	IB$_c$	4.20	3.81
ZAPS	IB$_b$	5.47	3.75
ZAPS	IIB$_c$	3.68	3.45
ZAPS	IIB$_b$	4.66	4.08

*Anaconda data is 1976 from most recent tapes available. ZAPS data is 1977.

‡Small subletters refer to probe location.

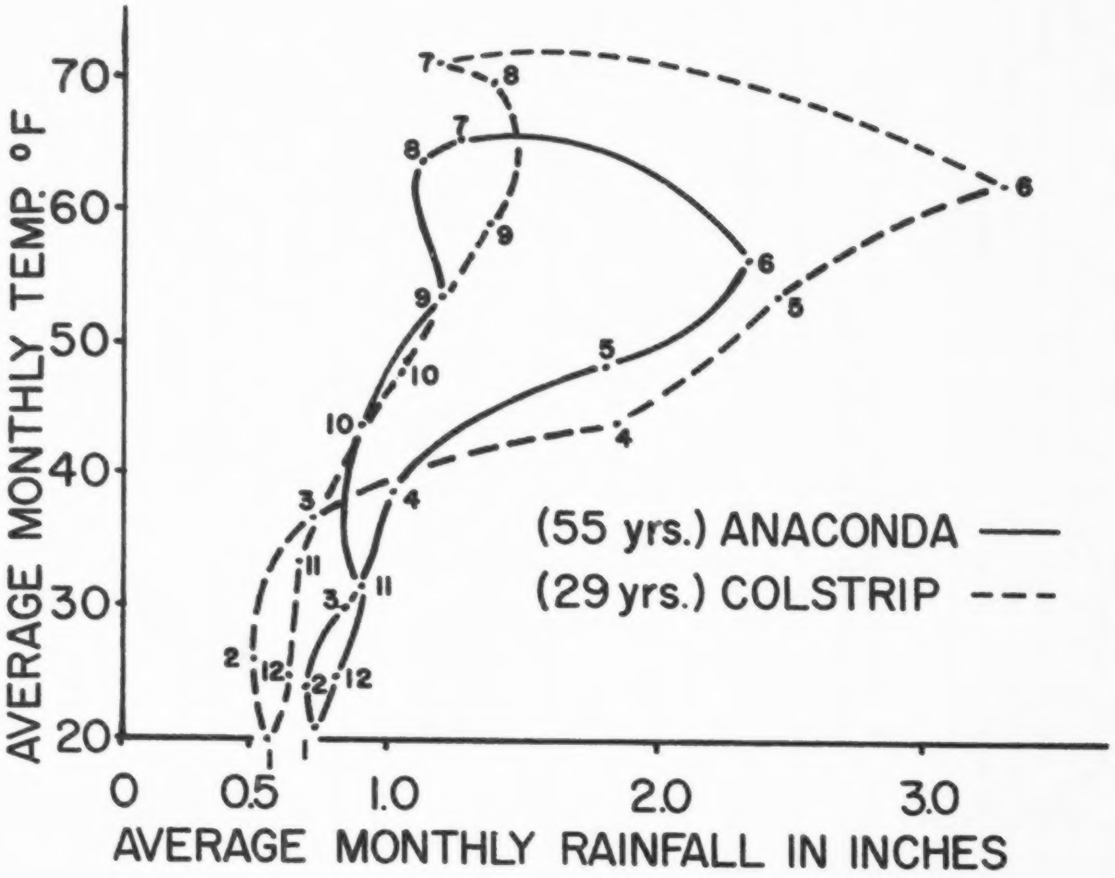


Figure 14.42. Climatograph for Anaconda and Colstrip; number indicates month of year.

Washing Experiment

The data derived from the simulated "rainfall event" (three-minute wash) and "overwintering" (four-day soak) tests are portrayed in Table 14.8. Only one sample of live *A. smithii* was of sufficient volume for washing. Of the six trials with multiple samples, only the data from the "rainfall event" with dead *K. cristata* had a t value with a probability greater than .10. This non-significant value may have resulted from unrepresentative division of the limited number of dead *K. cristata* culms available for the split in this one set of samples. The measured sulfur levels in some of these washed samples were considerably higher (as much as 1,500 ppm) than their "paired" unwashed samples, indicating that the older and younger leaves were not evenly split, and the variance increased accordingly.

TABLE 14.8. REDUCTION OF PLANT SULFUR LEVELS BY SIMULATED RAINFALL EVENT AND OVERWINTERING

$\bar{x}$ ppm Sulfur		$\bar{D}$	%D		paired-t	P
A. smithii--Live						
Unwashed/Washed						
2,825	2,117	-708	-25%	1	--	--
A. smithii--Dead						
Unwashed/Washed						
4,135	3,775	-360	-9%	10	2.518	$P \leq .025$
Unwashed/Soaked						
4,135	975	-2,225	-54%	10	4.678	$P \leq .005$
4,100	639	-3,461	-85%	9	11,946	$P \leq .001$
K. cristata--Live						
Unwashed/Washed						
3,475	2,787	-688	-20%	4	1.775	$P \leq .10$
K. cristata--Dead						
Unwashed/Washed						
2,615	2,593	-21	-1%	7	.066	$P \leq .95$
Unwashed/Soaked						
2,615	721	-1,743	-67%	7	11.515	$P \leq .001$

Two sets of data are presented for dead *A. smithii* subjected to the overwintering treatment. The case with ten paired samples included an overwintered sampled with a sulfur content of 4,000 ppm. A test for outliers gave an r = .857 ($P < .001$) (Dixon and Massey, 1957). In either case, the reduction in sulfur was highly significant. "Simulated overwintering" caused an average sulfur reduction of 69%. The last sulfur analysis for dead

A. smithii from IID in 1976 was 4,135 ppm, while the first analysis of dead (overwintered) materials in the spring of 1977 was 1,312, a reduction of 68%. The samples overwintered in the laboratory began to decay, and this enhanced the reduction in tissue sulfur levels. Similar biological activity also occurs under the snow cover and during spring thaws but at a slower rate. This is the period of most active fungal and bacterial decay in semi-arid climates. Spring and early summer collections of dead plant tissue would be of limited value in assessing air pollution-caused increases in sulfur titre.

The "rainfall event" data suggest an average reduction of 14%. The large volumes of water used in this simulation exceeded those of any rainstorm, although the duration of actual rainfall events is longer. It would seem that rainfall before a sampling period would not substantially reduce the measured level of tissue sulfur (at least at D plot fumigation levels) because the major portion of the sulfur is either in the plant or strongly adhered to the cuticle.

Intensity of rainfall in Rosebud and Powder River counties tends to be weighted towards low volume storms. A cumulative frequency duration of storm size for the growing season (April, May, and June) is presented in Figure 14.43, demonstrating that 87.8% of the rainfall events are less than .5 inch. The summer (July, August, and September) pattern is similar with 91.1% of the storms less than .5 inch. These data are based on the period 1950 through 1976 at Colstrip, Montana, and are typical of southeastern Montana. Low intensity storms that do not establish a leaf-flushing action (such as T or trace storm) may actually increase sulfur levels in or on plant tissues because of the hygroscopic nature of SO_2.

Fluoride Uptake

Fluoride levels of plant tissue were determined to assess whether soil fluoride uptake was influenced by SO_2 fumigation. Only the mid-September, 1976, collection from IIA and IID was utilized. These data are summarized in Table 14.9. If α was chosen to be .10, three of the four cases compared would exhibit a significant increase in the variability of fluoride content of plant tissues from IID tissues in contrast to vegetation from IIA. This increased variance may reflect greater stress on transpiration and root uptake processes on IID. The comparison of mean fluoride levels for the two treatment plots did not generate any evidence to support a difference in this parameter.

Vegetative Phenology

The data derived from the 1977 vegetative phenology of *A. smithii* are summarized in Tables 14.10a and 14.10b. During any observation period, the total leaf number per hundred culms was quite similar across the OPC and ZAPS I plots (Figure 14.44). No significant differences were observed among these five treatment plots. The leaf counts from the five plots were combined and a Gompertz curve fit to describe the progression of leaf stage development from May through September (Figure 14.45). Leaf stage development was rapid from mid-May until mid-July when growth plateaued at an average of 5.8 leaves per culm. A few culms developed additional leaves during late July and August but made no substantial contribution to final leaf stage (5.9 leaves per culm).

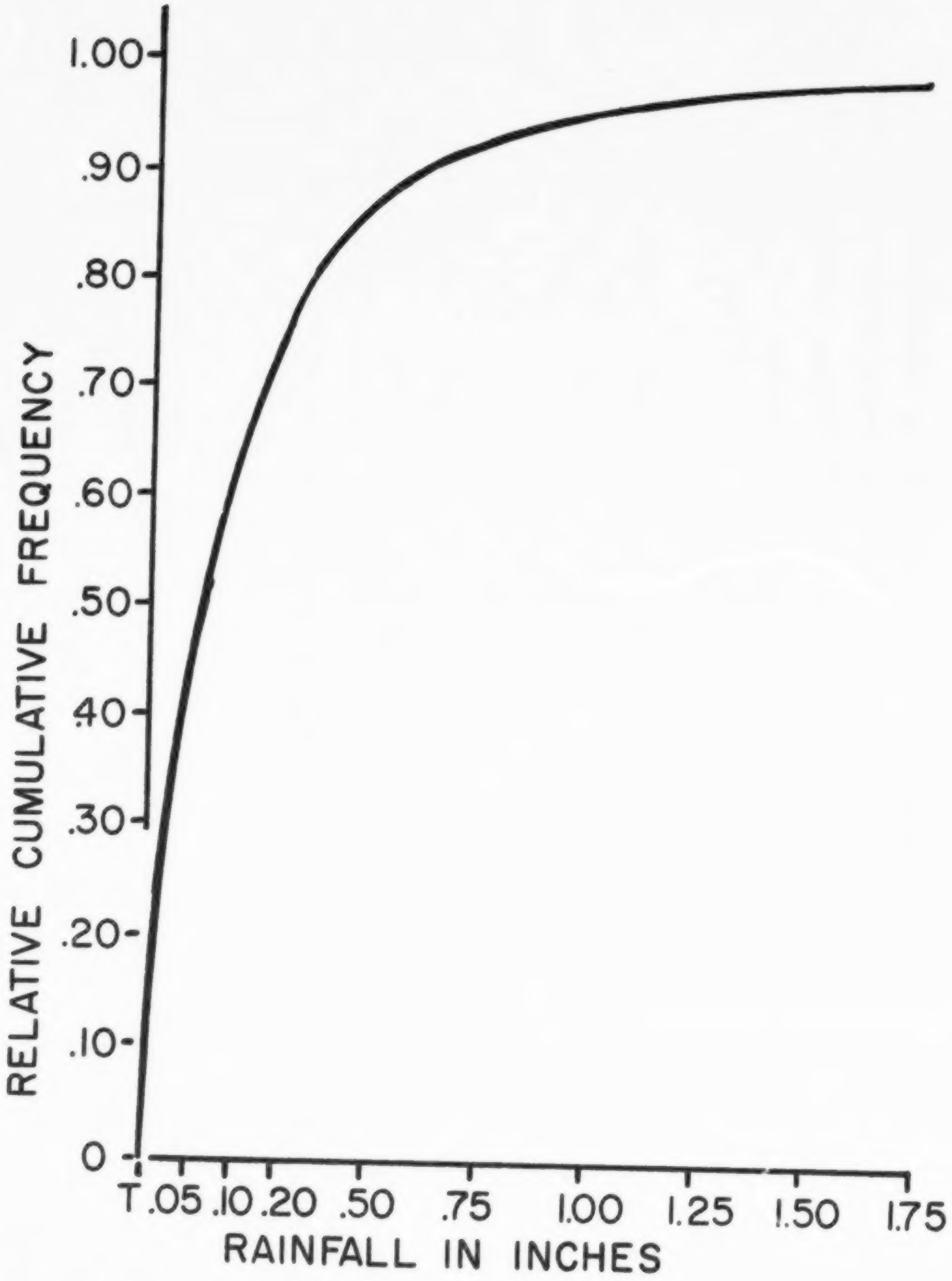


Figure 14.43. Typical distribution of storms by size in southeastern Montana.

TABLE 14.9. SOIL FLUORIDE UPTAKE AS INFLUENCED BY SO_2 FUMIGATION

	IIA				IID			Variance Ratio		Equality of Means*	
Species	$\bar{x}$ ppm	n	s^2		$\bar{x}$ ppm	n	s^2	$F = s_1^2/s_2^2$	P (exact)	F_s	P
K. cristata											
Live	2.09	8	.576	vs	2.73	10	2.725	4.731	.037	1.189	$.25 < P < .50$
Dead	3.00	7	.540	vs	4.13	6	2.131	3.946	.095	2.954	$.10 < P < .25$
A. smithii											
Live	1.78	9	.309	vs	2.07	6	1.367	4.424	.047	0.321	$.50 < P < .75$
Dead	4.07	10	5.791	vs	2.87	7	3.936	1.471	.625	1.262	$.25 < P < .50$

*Approximate test of $\mu_1 = \mu_2$ when $\sigma_1^2 \neq \sigma_2^2$; P based on synthetic degrees of freedom (Snedecor, 1956).

TABLE 14.10a. 1977 VEGETATIVE PHENOLOGY OF *AGROPYRON SMITHII* AND TEST FOR INDEPENDENCE OF LEAF NECROSIS ON ZAPS I

Observation	1	2	3	4	5	6	7	8	9
Date	12,13,14 May	28,29 May	15,16,17 Jun	2,3,4 Jul	12,20 Jul	4,5,6 Aug	22,23,24 Aug	10,11 Sept	28 Sept
OPC									
Total	307	383	471	557	582	584	590	589	592
Live	307	335	372	418	377	330	301	248	130
Necrotic	0	48	99	138	205	246	289	341	462
% Necrotic	0	12.5	21.0	24.8	35.2	42.1	49.0	57.9	78.0
IA									
Total	306	385	474	547	559	562	563	564	562
Live	306	348	365	389	312	271	238	164	65
Necrotic	0	37	109	158	247	291	325	402	497
% Necrotic	0	9.6	23.0	28.9	44.2	51.8	57.7	71.3	88.4
IB									
Total	305	382	485	572	610	616	619	619	619
Live	305	351	396	442	430	399	362	259	94
Necrotic	0	31	89	130	180	217	257	360	525
% Necrotic	0	8.1	18.4	22.7	29.5	35.2	41.5	58.2	84.8
IC									
Total	307	380	479	567	601	605	605	605	608
Live	307	332	356	393	356	325	290	209	51
Necrotic	0	48	123	174	245	280	315	396	557
% Necrotic	0	12.6	25.7	30.7	40.8	46.3	52.1	65.5	91.6
ID									
Total	304	377	467	541	581	590	592	592	591
Live	304	311	325	358	343	316	281	208	55
Necrotic	0	66	142	183	238	274	311	384	536
% Necrotic	0	17.5	30.4	33.8	41.0	46.4	52.5	64.9	90.7
Chi-square* Total Leaf No.	0.022	0.098	0.414	1.223	2.675	2.893	2.908	2.820	3.124
RXC Test of Independence[‡] G		18.184	22.169	25.983	33.990	36.780	33.983	31.093	59.877
1-P(G)		.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Maximal Nonsignificant Sets α ≤.05		D C O A B	D C A O B	D C A O B	A D C O B	A D C O B	A D C O B	A C D B O	C D A B O

*Critical chi-square $\chi^2_{.05[4]} = 9.488$

[‡] Source: Sokal and Rohlf, 1969

TABLE 14.10b. 1977 VEGETATIVE PHENOLOGY OF *AGROPYRON SMITHII* AND TEST FOR INDEPENDENCE OF LEAF NECROSIS ON ZAPS II

Observation Date	1 10,11,12 May	2 26,27 May	3 14,15 Jun	4 1,2 July	5 18,19 July	6 3,4 Aug	7 21,22 Aug	8 9,10 Sept	9 26,27,28 Sept
OPC									
Total	307	383	471	557	582	584	590	589	592
Live	307	335	372	418	377	338	301	248	130
Necrotic	0	48	99	138	205	246	289	341	462
% Necrotic	0	12.5	21.0	24.8	35.2	42.1	49.0	57.9	78.0
IIA									
Total	321	419	508	607	615	616	618	617	616
Live	321	367	384	446	360	264	199	91	39
Necrotic	0	52	124	161	255	352	419	526	577
% Necrotic	0	12.4	22.4	26.5	41.5	57.1	67.8	85.3	93.7
IIB									
Total	334	422	521	611	630	632	633	632	631
Live	334	367	394	446	393	304	250	147	90
Necrotic	0	55	127	165	237	328	383	485	541
% Necrotic	0	13.0	24.4	27.0	37.6	51.9	60.5	76.7	85.7
IIC									
Total	323	406	463	599	625	631	632	632	632
Live	323	354	336	417	375	310	260	186	122
Necrotic	0	52	127	182	250	321	372	446	510
% Necrotic	0	12.8	27.4	30.4	40.0	50.9	58.9	70.6	80.7
IID									
Total	326	414	523	648	680	695	695	694	694
Live	326	338	369	423	377	327	286	175	86
Necrotic	0	76	154	225	303	368	409	519	608
% Necrotic	0	18.4	29.4	34.7	44.6	52.9	58.8	74.8	87.6
Chi-square* Total Leaf No.	1.200	2.394	6.446	6.994	7.965	10.337*	9.339	9.347	8.998
RXC Test of Independence‡ G		8.438	10.890	18.363	13.475	29.050	44.984	122.980	77.528
1-P(G)		.077	.028	.001	.009	< .001	< .001	< .001	< .001
Maximal Nonsignificant Sets $\alpha \leq .05$		D B C O A	D C A B O	D C B A O	D A C B O	A D B C O	A B C D O	A B D C O	A D B C O

*Critical chi-square $\chi^2_{.05[4]} = 9.488$

‡Source: Sokal and Rohlf, 1969

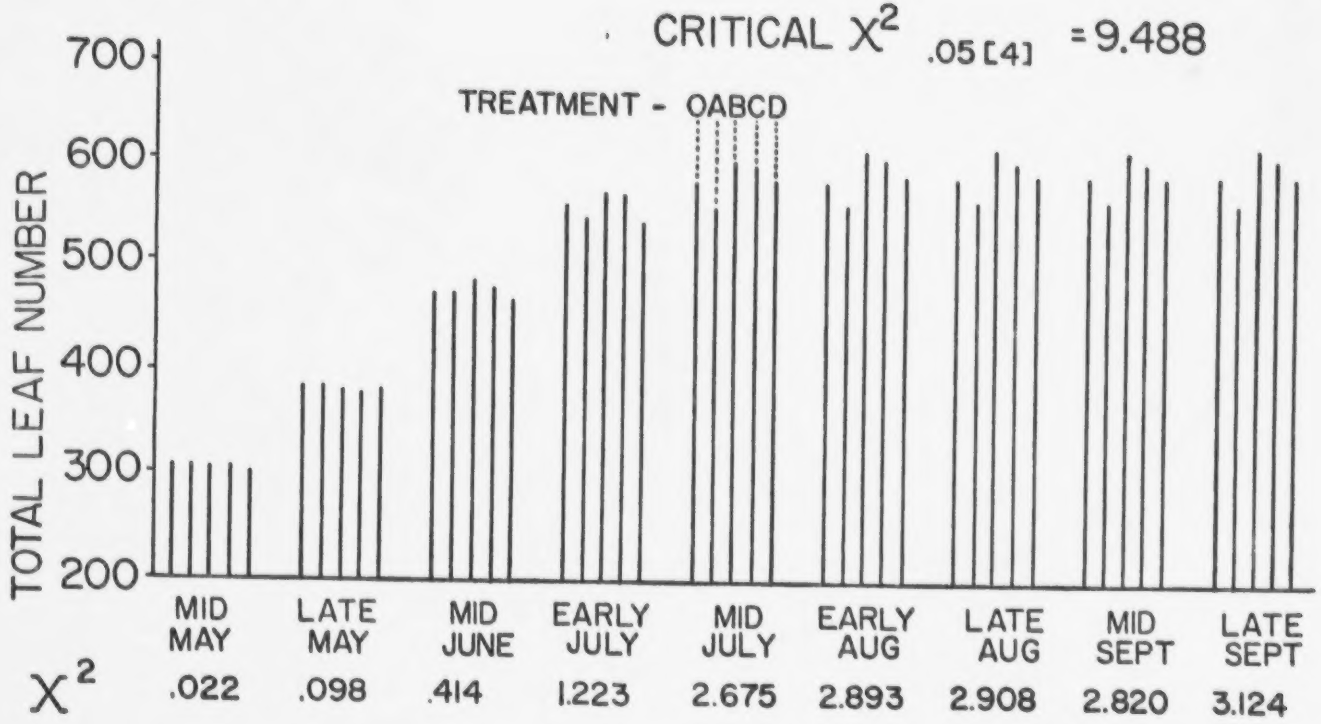


Figure 14.44. Total leaf number per hundred culms for *Agropyron smithii* on ZAPS I and OPC during 1977. Critical $\chi^2_{.05(4)} = 9.488$; treatments are ordered OPC, A, B, C, D for each observation period.

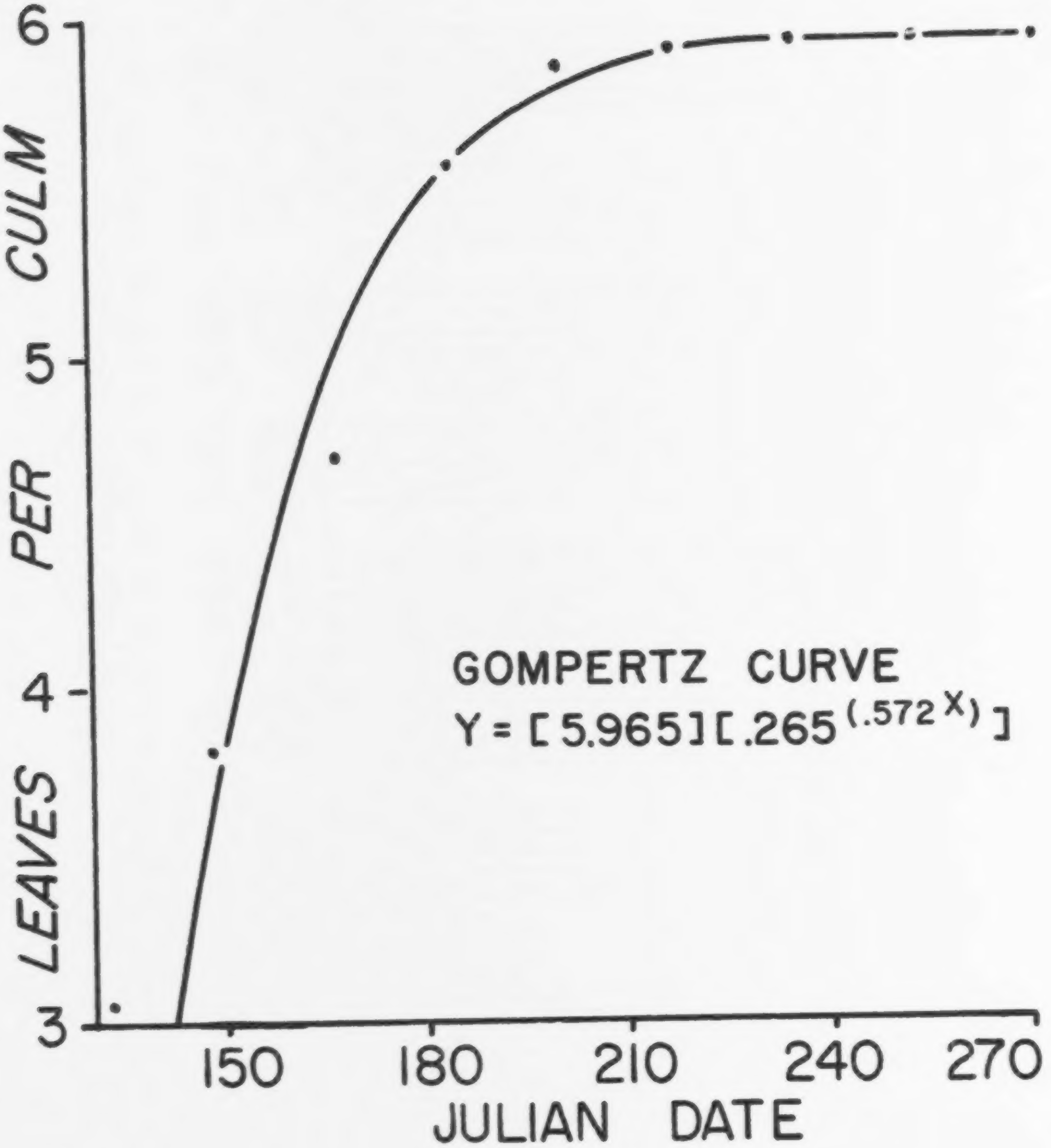


Figure 14.45. Development of *A. smithii* leaf stage number for the OPC and ZAPS I in 1977.

Leaf number on IID was higher than on the rest of the ZAPS II plots and the OPC (Table 14.10b). By early August, 6.9 leaves per culm was the average on IID, while OPC = 5.8, IIA = 6.12, IIB = 6.3, and IIC = 6.3 (Figure 14.46). The timing of development on ZAPS II was similar to that on ZAPS I plots, with IID showing a trend for further development.

Leaf stage development in 1977 was similar to the pattern observed in 1976 (TIR): rapid growth until mid-July and a gradual reduction during the rest of the season. The average leaf number on all plots was 6.27 in 1976, as opposed to 6.16 in 1977. However, no significant differences across the plots were measured in 1976.

The amount of leaf necrosis (ratio necrotic leaves to green leaves) across the treatment plots is summarized in the last row of Tables 14.10a and 14.10b. Doubling the sample size in 1977 to 100 plants per treatment plot increased the resolution across the plots but did not produce a significant separation of all the treatment plots. During the period of rapid growth (through mid-July, observation 5), significant premature senescence was most apparent on the plots receiving the higher levels of fumigation. During late May on ZAPS I (observation 2), the ID plot had significantly more necrotic leaves than IA and IB. The OPC, IA, IB, and IC plots were statistically indistinguishable.

During mid-June, the ID plants were also significantly more necrotic, this time in contrast to OPC and IB, while OPC, IA, IB, and IC were again statistically similar. By early July (observation 4), *A. smithii* on ID was more necrotic than on the OPC and IB plots, and the plants on IC showed a significant increase in necrotic leaves in contrast to IB. The OPC, IA, and IB plots continued to be inseparable as a group.

A second process became evident by late July (observation 5). The number of necrotic leaves increased rapidly on the IA plot after plant growth ceased. This plot was similar to IC and ID but exhibited significantly more necrosis than the OPC and IB. An identical relationship was evident through early August (observation 6). By late August (observation 7), all plots but the OPC showed less necrosis than IB, the OPC being similar to IB. In early September, IA, IC, and ID remained indistinguishable from each other. The plants on IA showed more necrosis than those on IB or the OPC, and on IC had more necrotic leaves than the OPC. By the end of September (observation 9), the IC and ID plots exhibited more necrosis than IB and the OPC, and IA showed more necrosis than the OPC.

In conclusion, the level of necrosis was highest on the IC and ID plots, as one might expect. The level of necrosis upon cessation of growth was higher on IA than might be anticipated. Although it was not significant, this pattern of necrotic development was observed on ZAPS I in 1976 (TIR).

During 1977 on ZAPS II, plots IIB and IID remained statistically indistinguishable throughout the collection season. In mid-June (observation 3), plants on IID were significantly more necrotic than those on the OPC. In early July (observation 4), IID showed more necrosis than both the OPC and IIA. Starting in mid-July (observation 5), the level of necrosis seemed to be

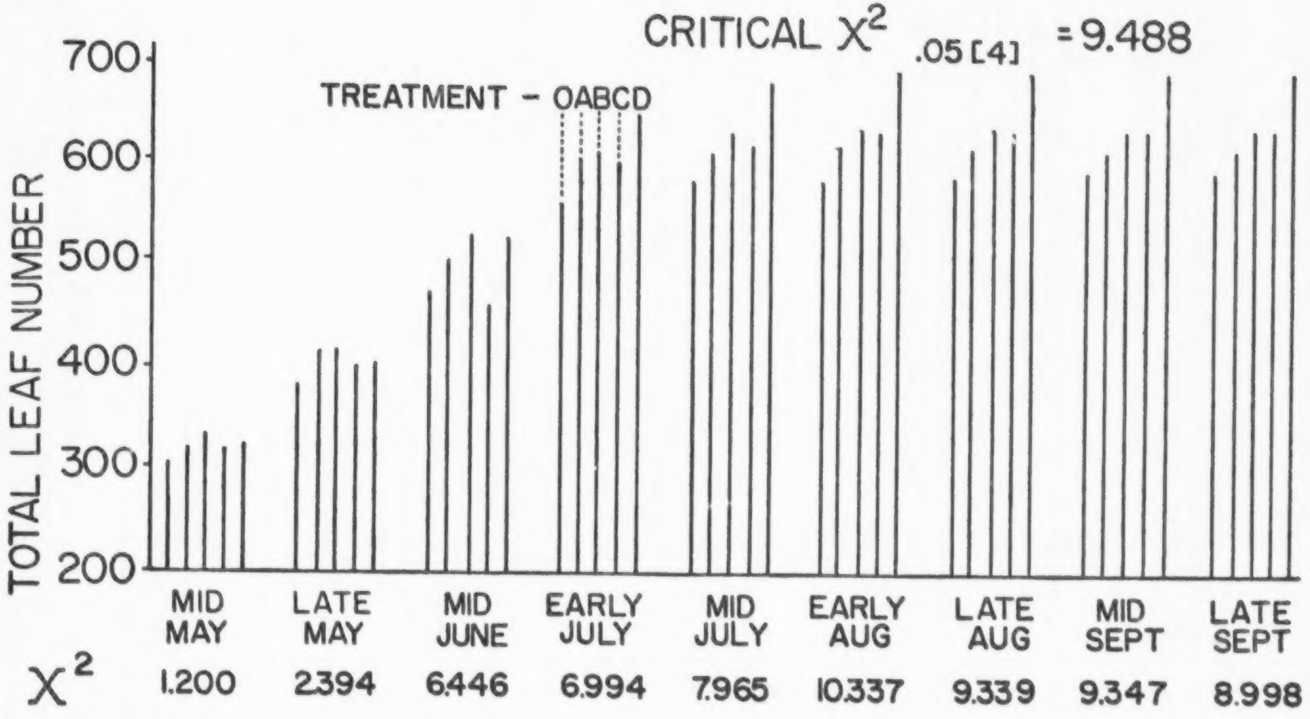


Figure 14.46. Total leaf number per hundred culms for *Agropyron smithii* on ZAPS II and OPC during 1977. Critical $\chi^2_{.05(4)}$ = 9.488; treatments are ordered OPC, A, B, C, D for each observation period.

increasing on the IIA plot, following the pattern on ZAPS I. Vegetation on IID was still more necrotic than vegetation on the OPC. In early August (observation 6), necrosis continued to increase on IIA, the only significant difference being that the OPC was less necrotic than IIA, IIB, and IID. By late August (observation 7), the amount of necrosis was significantly higher on IIA than on IIC, IID, or the OPC; vegetation on the OPC displayed significantly less necrosis than on all other plots. In early September (observation 8), necrosis was most prevalent on IIA and least prevalent on the OPC. There was no detectable difference between IIB, IIC, and IID at this time. The final observation period (9) in mid-September showed that the largest amount of necrosis occurred on IIA, this plot being significantly elevated over all other treatments. Plot IID was more necrotic than IIC or the OPC, and IIB was also more necrotic than the OPC.

The overall pattern on ZAPS II was one of greatest necrosis on vegetation on the most fumigated plot and least necrosis on IIA and OPC vegetation through the period of leaf development and expansion. After growth ceased, the vegetation on IIA began to become necrotic at a faster rate than vegetation on the other treated plots. The pattern was similar on ZAPS I, with the exception of the intermediate to low level of necrosis displayed by vegetation on IB. On ZAPS II in 1976, the IIA plot exhibited a similar but non-significant pattern of low level necrosis early in the season but showed a relatively high amount of necrosis late in summer.

Seed Work

A. smithii germination was so low as to preclude data analysis. A total of 5,113 seeds from all treatments exhibited a total germination success of only 0.12% after wet stratification under dark conditions at 4°C for 30 days. The germination test was conducted at an alternating 20°C/5°C diurnal cycle under dark conditions. The stratification and germination conditions were recommended by Eddleman who also has found limited seed fertility in *A. smithii* (1977b). To ascertain whether the low germination success was a consequence of continued dormancy or improper germination environment, a tetrazolium test was made on seeds from IA. Seeds selected for fill (presence of embryo) were soaked at 35°C for two hours, then bisected longitudinally. No viable embryos were detected after 20 hours staining in 0.1% tetrazolium at 35°C. Approximately 30% of the caryopses were occupied by insect bodies which had exhausted the endosperm. The lack of germination success appears to be due to a lack of viable embryos.

Lack of viable seed is typical of *A. smithii*. Jorgensen (1970) reported germination success ranging from 0% to 4.0% on eleven sites in central Montana over a two-year period. *A. smithii* reproduction tends to be primarily by vigorous rhizome growth. Nevertheless, seed production is still important. New seed maintains the vitality of a stand and allows long-term changes in the population. Germination success as high as 80% (Hoover, *et al.*, 1948) has has been reported in good seed crop years.

Germination success results for *Stipa viridula* are reported in Tables 14.11 and 14.12. Germination success was quite low, ranging from 0.6% to 3.9%, and no significant treatment effects were noted. The data on seed weights

TABLE 14.11 SEED GERMINATION CRITERIA

Criteria	*A. smithii*	*S. viridula*	*K. cristata*	*P. sandbergii*	*T. dubius*
*Collection Date	17, 19 Sept	6 August	6 July	4, 7 July	15 July
Recommended Date		7-17 July	1-30 July	10 June-17 July	
*Average Weight Range (grams x 10^{-4}/seed)	2.58- 3.35 x 10^{-3}	1.19- 2.06 x 10^{-3}	1.90- 2.50 x 10^{-4}	3.19- 4.73 x 10^{-4}	6.04- 7.58 x 10^{-3}
1976 Average Weight (grams x 10^{-4}/seed)		3.47 x 10^{-3}	2.03 x 10^{-4}	4.58 x 10^{-4}	
*Days to 50% Germination			6-7	10-11	5-8
Days to 50% Germination		5	5	12	
*Range of % Germination	0-0.6	0.6-3.9	32.9-55.2	21.9-56.5	66.1-94.7
1976 Average % Seed Fill and Range		89 (78-100)	65 (33-91)	78 (65-90)	
After Ripening	No	12 mo	4 mo	4 mo	No
Stratification	30 days, 4°C, dark	No	No	No	No
Germination Temperature Day/Night (°C)	20°/5°	20°	20°	10°	20°
Hours Light	No	No	No	8	No

*Data for 1976 ZAPS seed collections; all other data and recommendations from Eddleman (1977a, 1977b).

TABLE 14.12. PERCENT GERMINATION, PERCENT VIABILITY, AND SEED WEIGHTS FOR 1976 COLLECTIONS FROM ZAPS

Species	Plots*			
Stipa viridula				
Percent Germination	IA 0.7	IC 1.6	IB 1.8	ID 1.8
Seed Weight (grams x 10^{-3}/seed)	IA 1.57	IB 2.02	ID 2.02	IC 2.06
Percent Germination	IIC 0.6	IID 0.8	IIA 2.4	IIB 3.9
Seed Weight (grams x 10^{-3}/seed)	IIA 1.19	IID 1.30	IIC 1.47	IIB 1.63
Koeleria cristata				
Percent Germination	ID 32.9	IC 48.7	IA 52.6	IB 55.2
Percent Viable	ID 18.4	IA 40.2	IC 40.6	IB 56.9
Percent Viable (selected)	ID 17.2	IC 35.1	IA 41.9	IB 52.4
Seed Weight (grams x 10^{-4}/seed)	ID 1.90	IA 2.31	IB 2.42	IC 2.50

*Underlined subsets do not differ significantly ($P \leq .05$) (Duncan's Multiple Range Test).

(continued)

TABLE 14.12. (continued) PERCENT GERMINATION, PERCENT VIABILITY, AND SEED WEIGHTS FOR 1976 COLLECTIONS FROM ZAPS.

Species	Plots*				
Poa sandbergii					
Percent Germination	ID	IC	IA	IB	
	39.2	53.0	53.3	56.5	
Seed Weight (grams x 10^{-4}/seed)	ID	IC	IB	IA	
	3.66	4.05	4.63	4.73	
Percent Germination	IID	IIA	IIB	IIC	
	21.9	25.1	25.9	44.0	
Seed Weight (grams x 10^{-4}/seed)	IIA	IID	IIB	IIC	
	3.19	3.41	3.75	4.33	
Tragopogon dubius					
Percent Germination	IA	OPC	IB	IC	ID
	66.1	80.9	87.8	88.6	91.0
Seed Weight (grams x 10^{-3}/seed)	ID	IA	IC	OPC	IB
	6.04	6.76	6.85	6.95	7.58
Percent Germination	OPC	IIB	IIC	IIA	IID
	80.9	84.2	85.1	90.2	94.7
Seed Weight (grams x 10^{-3}/seed)	IID	IIC	OPC	IIA	IIB
	6.67	6.69	6.95	6.98	6.98

*Underlined subsets do not differ significantly ($P \leq .05$) (Duncan's Multiple Range Test).

suggest a depression in average weight on the control plots. These data are suspect. Eddleman (1977b), reporting that July 12 is the average date for the peak of ripe seed and that 50% shatter occurs by July 17, comments, "There appears to be little more than a week for optimum collection." Our 1976 collections were made quite late (August 6) and seem to consist almost entirely of seed that never matured. Eddleman's (1977b) average seed weight for 1976 was 3.47×10^{-3} grams, while our average was from 1.19 to 2.06×10^{-3} grams. At the time of collection, we felt we had missed the shatter but were not fully aware of the consequences. Under the recommended germination conditions (20°C, dark, no stratification, approximately one year after ripening period), germination success should have been much higher. The limited total germination also precluded the calculation of rate of germination parameter.

The *K. cristata* collection from ZAPS I was made on July 6. This date is in agreement with that recommended (Table 14.11), and the range of seed weights includes the regional average for that year. Germination testing was carried out at 20°C under dark conditions. A significant depression in germination success was observed for seeds collected from the D plot (Table 14.12). A corresponding reduction in seed weight was also observed.

Tetrazolium viability tests were conducted to confirm the germination test. The seeds were soaked for 24 hours at room temperature. Soaking softened the seeds and prevented splitting when the endosperm was pierced by a needle. Piercing allows rapid absorption of the tetrazolium solution. The replicates (five per treatment, 20 seeds per replicate) were then treated with 1.0% tetrazolium at 35°C for 24 hours. The stained seeds were then treated with lactophenol for one hour to make the palea transluscent and allow inspection of the embryo. A significant reduction in viability (Table 14.12) was again noted in seeds from ID, confirming the observed reduction in germination success.

A third test was made on *K. cristata* seeds to select seeds for fill. This was done by backlighting the seeds on a light table. After these seeds were stained and cleared (lactophenol), a notable pattern in insect infestation was seen (see Bromenshenk, Section 18). Under backlighting, the dark-bodied insects appeared similar to seed embryos. The seeds containing insects were removed from the subsequent data analyses. Again, a significant depression in viability was observed in seeds from plot ID (Table 14.12). The IC plot was also found to be significantly depressed relative to IB.

The proportion of viable embryos would be expected to be equal to or greater than the proportion of seeds showing successful germination. The data suggest a lower proportion of viable seeds than the germination test. The most direct explanation may be that the viability tests were made four months after the germination test. The extra time and possible storage environment stresses may have reduced viability of these seeds. Although the trials were not designed to test this hypothesis, anova was performed for the same plots, contrasting the germination success with the percent viable and the percent viable (selected). The resultant F values are presented in Table 14.13. Seeds from IC and ID seem to be becoming nonviable at a faster rate than those from IA and IB.

TABLE 14.13. F VALUES FROM ANOVA OF GERMINATION SUCCESS AND VIABILITY TESTING

Plot	% Germination vs % Viable	% Germination vs % Viable (selected)	% Viable vs % Viable (selected)
ID	20.090*	22.186*	0.182
IC	1.380	10.262*	0.898
IB	0.109	0.419	0.739
IA	4.429	3.510	0.084

*$P < .05$

Both viability trials were performed at the same time. If we were able to effectively select for fill by the backlighting technique, we might expect to see an increase in percent viable. No significant changes in viability were observed (Table 14.13). This suggests that almost all the seeds contained an embryo, and the observed depression in germination success and viability across the treatment occurred after the formation of the embryo. Therefore, SO_2 fumigation may not have prevented embryo development itself. Possible fill identification problems under backlighting and the limited data base caution against drawing either of these conclusions at this time.

The large numbers of germinating *K. cristata* seeds allow a detailed examination of the pattern and speed of germination of this species. Germination was rapid and intense (Figure 14.47), with the day of peak germination (day 6) being the same for all treatment plots. Note: In Figures 14.47 to 14.52, the proportion of seeds successfully germinating is reiterated in the body of the graph, while the vertical axis portrays the relative frequency of those seeds which did germinate. There were six days to 50% germination in seeds from IA and IB, while seeds from IC and ID required more time (Table 14.11). The response of seeds from both ZAPS sites in 1976 was slower than that reported for the region (5 days), but the difference was not significant.

The relative cumulative frequencies (Table 14.14) allow a detailed examination of the speed of germination over the entire 30-day period. The response was fastest on IA and IB and significantly slower on IC and ID. On days 6 and 7, ID was also lagging significantly behind IC. The entire time period is integrated in the coefficient of rate of germination (CRG $= \sum_{1}^{n}[g_n - g_{(n-1)}]/n$). This coefficient was not subjected to statistical analyses, but it can be seen to decrease with increasing levels of fumigation.

Poa sandbergii seeds were collected on July 4 from ZAPS I and on July 7 from ZAPS II, within the recommended period (Table 14.11). The range of observed seed weights are typical of this species. Germination trials were

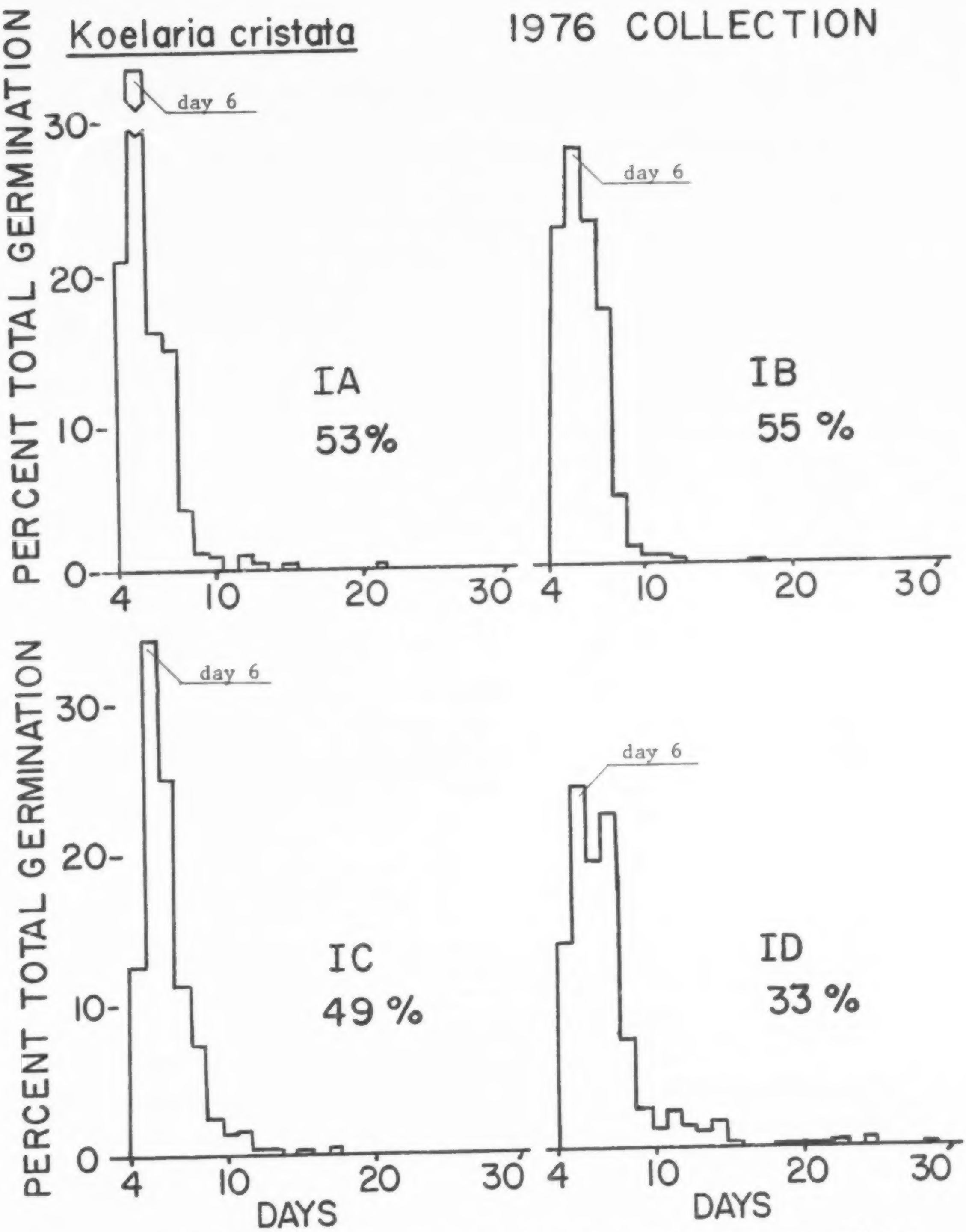


Figure 14.47. Germination peak and distribution for *Koelaria cristata* collected in 1976 from ZAPS I.

TABLE 14.14. RELATIVE CUMULATIVE FREQUENCIES FOR GERMINATION OF *KOELERIA CRISTATA* DURING 1976 ON ZAPS I

Day	Plot*			
	IA	IB	IC	ID
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	21.4^{ef}	23.0^{e}	12.8^{g}	13.9^{fg}
6	58.8^{e}	51.5^{ef}	47.8^{f}	38.4
7	75.1^{e}	73.8^{e}	73.4^{e}	57.9
8	90.2^{e}	91.1^{e}	84.9^{ef}	80.7^{f}
9	94.6^{e}	95.8^{e}	92.4^{e}	87.9^{e}
10	96.1^{e}	97.1^{e}	95.0^{e}	90.5^{e}
11	97.2^{e}	97.8^{e}	96.5^{e}	91.7^{e}
12	" e	98.5^{e}	98.2^{e}	94.1^{e}
13	98.3^{e}	99.0^{e}	98.6^{e}	95.5^{e}
14	98.8^{e}	99.2^{e}	97.0^{e}	96.5^{e}
15	99.0^{e}	" e	"	98.2^{e}
16	99.4^{e}	" e	99.4^{e}	98.4^{e}
17	" e	" e	" e	" e
18	" e	99.4^{e}	100.0^{e}	" e
19	-	-	-	-
20	-	-	-	-
21	-	-	-	-
22	99.8^{e}	99.9^{e}	" e	98.9^{e}
23	100.0^{e}	" e	" e	99.1^{e}
24	" e	" e	" e	" e
25	" e	" e	" e	99.6^{e}
26	" e	" e	" e	" e
27	-	-	-	-
28	-	-	-	-
29	" e	" e	" e	99.8^{e}
30	" e	" e	" e	" e
CRG	15.65	15.56	14.96	14.23
n	546	548	469	416

*Treatment-days within a row sharing the same letter do not differ significantly ($P \overset{\sim}{\leq} .05$) (modified Smirnov Test).

conducted at 10°C with an eight-hour light period for 30 days. No tetrazolium assessments of viability were made.

The success of germination was significantly reduced on ID (Table 14.12), in comparison to the other three ZAPS I plots. This reduction in percent germination was paralleled by a reduction in the weights of seeds from the plots receiving higher SO_2 fumigation: IC and ID were significantly lower than IA and IB.

As with *K. cristata*, the total number of *P. sandbergii* seeds germinating was large enough to allow examination of the pattern of germination response through time. Germination in the species was rapid (Figure 14.48). Day of peak germination was 11 for all four treatment plots, but a less intense peak was observed on day 9 in the seeds from IA. Days to 50% germination are around 12 for this species and occurred on day 11 for all plots in these trials (Table 14.11).

The relative cumulative frequency distributions are presented in Table 14.15. Significant differences were observed for days 8 through 11. Plot IA exhibited the most rapid rate of germination, and IC was the slowest. Plots IB and ID were similar through the entire time period but were significantly slower than IA on some days. The CRG reveals a similar but less detailed pattern. The speed of germination was greatest on IA, reduced but similar on IB and ID, and slowest on IC.

P. sandbergii seed from ZAPS II was germinated under conditions identical to those from ZAPS I. Plot IID showed the lowest germination success, although the difference was not significant, while total germination success was significantly higher on IIC (Table 14.16). The weight data revealed IIC seeds to be heavier than those from IIA and IID but not from IIB.

Analyses of the germination pattern through time revealed a depression corresponding to higher fumigation levels in spite of the higher germination success on plot IIC. Day of peak germination was 11 on plots IIB, IIC, and IID, while IIA peaked a day earlier (Figure 14.49). Additionally, the peak for IID appears truncated, with relatively more germination occurring late in the trial. This was reflected by the fact that 50% of final germination was reached on day 11 by seeds from IIA, IIB, and IIC but not until day 13 on IID.

The relative cumulative frequency distributions (Table 14.16) show that the plot IIA sample had the most rapid germination. The speeds of germination on IIB and IIC were similar, but both were significantly slower than that of IIA. Response speed on IID was drastically reduced in comparison to the other three treatments. The relative magnitude of the CRG again integrates the overall trend, with speed of germination decreasing with increasing level of fumigation.

Tragopogon dubius was the only forb used in our seed germination studies. This plant is a diploid weed of European origin which can establish itself in areas which are relatively undisturbed (Hitchcock and Cronquist, 1973). The studied grasses were all perennials, but *T. dubius* acts as an annual, biennial, or winter annual (Hitchcock and Cronquist, 1973; Van Bruggen, 1976) and

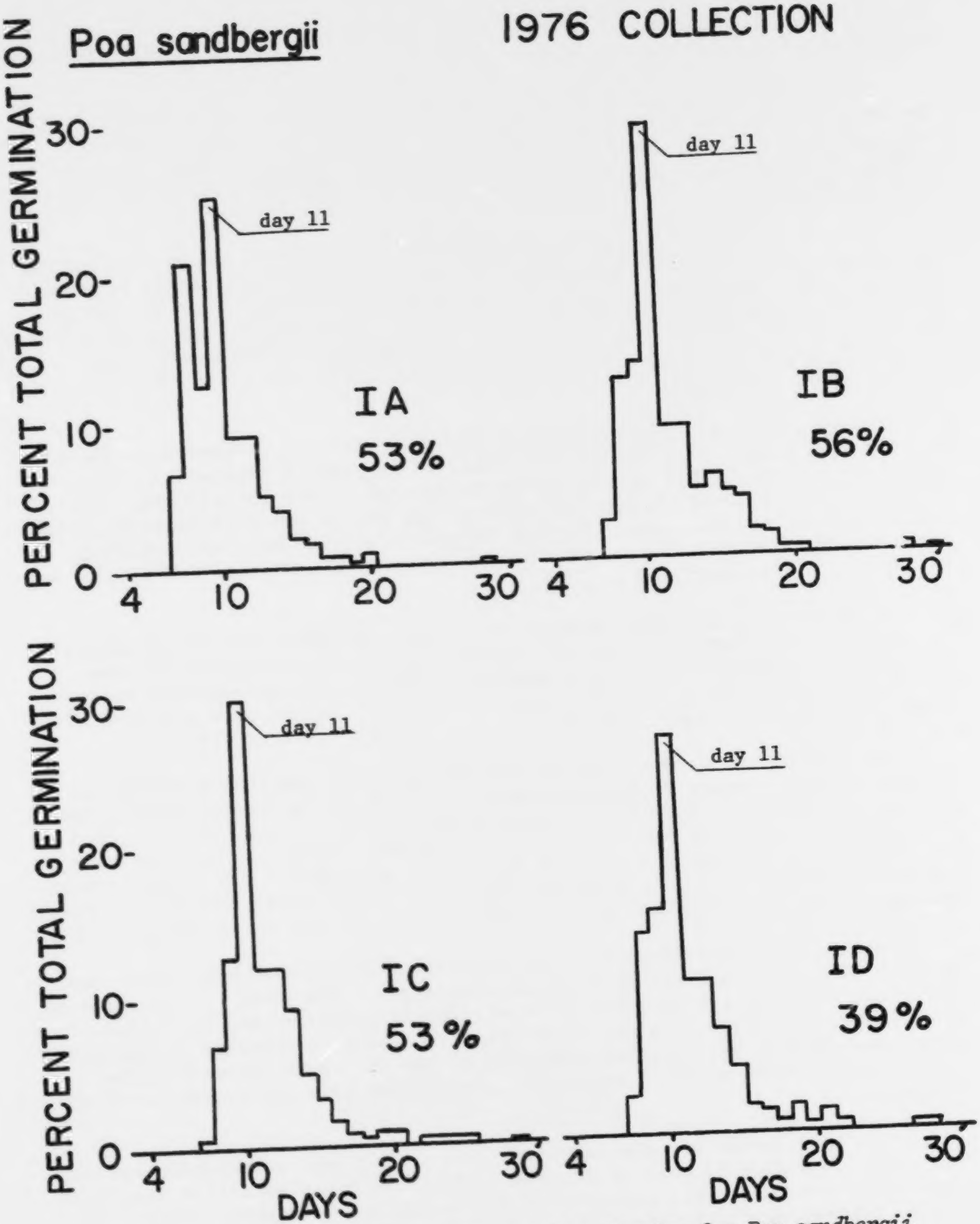


Figure 14.48. Germination peak and distribution for *Poa sandbergii* collected in 1976 from ZAPS I.

TABLE 14.15. RELATIVE CUMULATIVE FREQUENCIES FOR GERMINATION OF *POA SANDBERGII* DURING 1976 ON ZAPS I

	Plot*			
Day	IA	IB	IC	ID
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	6.6 ^{e}	2.4 ^{e}	.5 ^{c}	2.6 ^{e}
9	27.8	14.6 ^{e}	7.5 ^{e}	16.5 ^{e}
10	40.5 ^{e}	28.0 ^{fg}	20.5 ^{f}	31.0 ^{eg}
11	65.2 ^{e}	57.8 ^{ef}	51.2 ^{f}	58.0 ^{ef}
12	-	-	-	-
13	83.2 ^{e}	75.8 ^{e}	75.6 ^{e}	79.0 ^{e}
14	88.2 ^{e}	80.4 ^{e}	84.9 ^{e}	86.1 ^{e}
15	92.2 ^{e}	86.0 ^{e}	89.9 ^{e}	90.6 ^{e}
16	94.3 ^{e}	90.4 ^{e}	93.1 ^{e}	92.5 ^{e}
17	96.3 ^{e}	94.5 ^{e}	94.9 ^{e}	94.1 ^{e}
18	97.0 ^{e}	96.2 ^{e}	95.8 ^{e}	94.7 ^{e}
19	97.8 ^{e}	97.7 ^{e}	96.5 ^{e}	96.9 ^{e}
20	98.1 ^{e}	98.2 ^{e}	97.4 ^{e}	97.1 ^{e}
21	98.9 ^{e}	98.7 ^{e}	98.3 ^{e}	98.7 ^{e}
22	98.9 ^{e}	98.7 ^{e}	98.3 ^{e}	99.2 ^{e}
23	-	-	-	-
24	-	-	-	-
25	-	-	-	"
26	99.7 ^{e}	99.2 ^{e}	99.9 ^{e}	99.5 ^{e}
27	"	"	"	99.8 ^{e}
28	"	99.7 ^{e}	"	"
29	100.0 ^{e}	"	100.1 ^{e}	"
30	"	99.9 ^{e}	"	
CRG	9.13	8.57	8.35	8.69
n	377	410	440	310

*Treatment-days within a row sharing the same letter do not differ significantly ($P \lesssim .05$) (modified Smirnov Test).

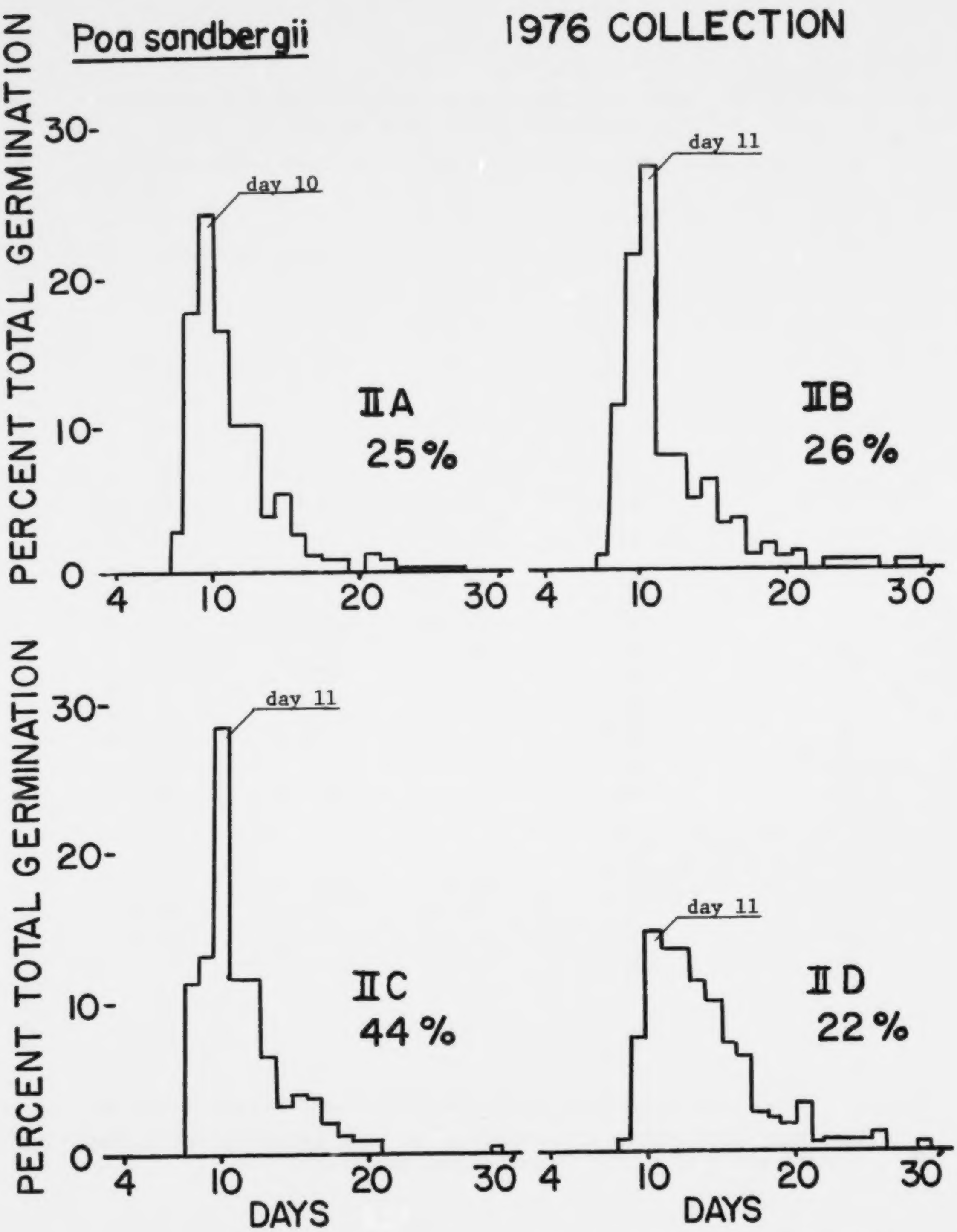


Figure 14.49. Germination peak and distribution for *Poa sandbergii* collected in 1976 from ZAPS II.

TABLE 14.16. RELATIVE CUMULATIVE FREQUENCIES FOR GERMINATION OF *POA SANDBERGII* DURING 1976 ON ZAPS II

	Plot*			
Day	IIA	IIB	IIC	IID
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	2.7^{e}	0.9^{e}	0^{e}	0^{e}
9	21.3^{e}	12.0^{ef}	11.5^{eg}	0.9^{fg}
10	45.9	33.4^{e}	24.8^{e}	8.6
11	62.2^{e}	60.8^{e}	53.9^{e}	23.6
12	-	-	-	-
13	82.3^{e}	76.2^{e}	77.5^{e}	50.9
14	86.1^{e}	81.0^{e}	84.1^{e}	62.3
15	91.4^{e}	87.0^{e}	87.3^{e}	71.4
16	94.1^{e}	90.1^{ef}	91.3^{e}	78.7^{f}
17	95.2^{e}	93.5^{e}	95.0^{e}	85.1^{e}
18	96.0^{e}	94.4^{e}	97.0^{e}	87.8^{e}
19	96.8^{e}	96.1^{e}	98.2^{e}	90.1^{e}
20	96.8^{e}	96.6^{e}	99.1^{e}	91.9^{e}
21	97.9^{e}	97.8^{e}	100.0^{e}	95.1^{e}
22	98.7^{e}	"	"	95.6^{e}
23	-	-	-	-
24	-	-	-	-
25	-	-	-	-
26	99.8^{e}	99.5^{e}	100.6^{e}	98.3^{e}
27	100.2^{e}	"	"	99.7^{e}
28	"	100.0^{e}	"	"
29	"	100.5^{e}	101.2^{e}	100.2^{e}
30	"	"	"	"
CRG	8.95	8.62	8.53	7.30
n	264	233	347	220

*Treatment-days within a row sharing the same letter do not differ significantly ($P \leq .05$) (modified Smirnov Test).

never as a perennial. This species also exhibited the exceptionally high levels of sulfur (TIR). Sulfur in leaves from the OPC averaged 4,108 ppm, while leaves from the ID plot averaged 7,575 in 1976. Much more extensive collections were made in 1977, but chemical analysis has not been completed.

T. dubius seeds were collected on July 15. Germination trials were conducted at 20°C under dark conditions. An OPC sample was available for inclusion in the trials. The IA plot displayed a significant reduction in germination success when compared to the other treatments (Table 14.17). The trial for the ZAPS II samples revealed no significant differences (Table 14.18). Seed weights from ZAPS II exhibited no difference, while ZAPS I seeds from the D plot were significantly lighter, and IB seeds were significantly heavier than those from the other three treatments.

The pattern of germination is displayed in Figures 14.50 through 14.52. Day of peak germination was day 5 for seeds from all plots, except IC (day 7) and IA (day 9). There was also a tendency for a biomodal response, but this could be an artifact of the limited (137 or less) total number of seeds germinating in each sample. Days to 50% germination also tended to be variable, ranging from day 5 to day 8 (Table 14.11), with no pattern corresponding to the level of SO_2 treatment.

The relative cumulative frequency distributions differed greatly (Tables 14.17 and 14.18), but the shift in speed of response did not correspond in any consistent way with plot treatment levels. Similarly, the CRGs were highly varied and followed no recognizable pattern. Within the limits of the sample size employed (replicates of four, total seed numbers from 198 to 497, germination numbers from 174 to 452), it appears that the seed of this species is independent of the level of SO_2 fumigation.

Mycorrhizal Studies

Regression of percent mycorrhizal infection on both absorbance (Figure 14.53) and theoretical SO_2 dosage (Figure 14.54) from collection 29 (mid-September) on ZAPS II was found to be significant at the $P \leq .001$ and $P \leq .05$ levels, respectively. Thus a large and significant portion of the percent mycorrhizal infection levels, as determined by visual examination, could be explained by theoretical SO_2 dosage and by spectrophotometer absorbance readings of the root extracts. The resultant trend, as seen in Figures 14.53 through 14.56 was for those plants from the high SO_2 dosage plots (C and D) to exhibit both low absorbance readings and relatively lower percent mycorrhizal infection levels.

Roots of *A. smithii* were not easily distinguishable in the field on the basis of color. However, when stained and microscopically examined, *A. smithii* root samples from the OPC collection 26 (mid-July) consisted of a larger number of fat roots with a yellow-gold coloration, compared to those from ZAPS IIC and IID. Roots from the higher treatment plots tended to have many more thin, dark brown roots. Until additional sampling and further analysis of absorbance data is conducted, however, the 95% confidence limits (Figure 14.53) indicate that absorbance readings alone do not provide the desired accuracy for prediction of percent mycorrhizal infection levels on the ZAPS.

TABLE 14.17. RELATIVE CUMULATIVE FREQUENCIES FOR GERMINATION OF *TRAGOPOGON DUBIUS* DURING 1976 ON ZAPS I

	Plot*				
Day	OPC	IA	IB	IC	ID
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	0	0	0	0	0
5	20.0^{e}	11.9^{ef}	23.6^{e}	7.2^{f}	55.8
6	37.1^{e}	29.6^{e}	39.1^{e}	15.4	85.7
7	56.5^{e}	43.9^{e}	54.6^{e}	31.4	90.6
8	62.8^{e}	55.2^{ef}	64.9^{e}	42.7^{f}	93.3
9	66.8^{e}	74.0^{e}	71.2^{e}	52.0	94.2
10	73.1^{e}	79.5^{e}	76.9^{e}	58.7	95.3
11	79.4^{e}	82.9^{e}	81.5^{e}	64.4	96.2
12	80.0^{e}	84.3^{ef}	83.8^{e}	73.7^{f}	96.4
13	82.3^{e}	86.7^{e}	87.2^{e}	76.8^{e}	96.8
14	84.6^{efh}	93.9^{eg}	94.7^{fgi}	78.9^{h}	97.2^{i}
15	85.7^{ef}	97.0^{e}	96.4^{eg}	80.4^{f}	97.4^{eg}
16	88.6^{efgh}	97.7^{f}	" gf	81.4^{h}	" ef
17	89.7^{ef}	98.0^{e}	" e	82.4^{f}	" e
18	91.4^{ef}	" e	" eg	83.9^{fg}	" e
19	93.1^{e}	98.7^{f}	" eg	85.4^{fgh}	" eh
20	94.8^{e}	" e	" e	86.9^{e}	97.6^{e}
21	96.5^{e}	99.0^{e}	97.0^{e}	" e	" e
22	" e	" e	" e	92.6^{e}	98.7^{e}
23	" e	" e	" e	97.8^{e}	99.1^{e}
24	–	–	–	–	–
25	–	–	–	–	–
26	–	–	–	–	–
27	98.2^{e}	" e	99.3^{e}	100.4^{e}	" e
28	99.3^{e}	99.3^{e}	" e	" e	" e
29	99.9^{e}	99.6^{e}	" e	" e	99.3^{e}
30	" e	" e	99.9^{e}	" e	99.5^{e}
31	" e	99.9^{e}	" e	100.9^{e}	99.9^{e}
CRG	13.25	12.90	13.73	11.72	17.67
n	175	293	174	194	452

*Treatment-days within a row sharing the same letter do not differ significantly ($P \tilde{\lesssim}$ .05) (modified Smirnov Test).

TABLE 14.18. RELATIVE CUMULATIVE FREQUENCIES FOR GERMINATION OF *TRAGOPOGON DUBIUS* DURING 1976 ON ZAPS II

Day			Plot*		
	OPC	IIA	IIB	IIC	IID
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	0	0	0	0	0
5	20.0^{e}	61.9^{f}	49.7^{f}	25.7^{e}	31.9^{e}
6	37.1^{e}	75.8^{f}	80.4^{f}	37.7^{e}	41.3^{e}
7	56.5^{e}	84.0^{f}	85.9^{f}	50.8^{e}	54.9^{e}
8	62.8^{e}	85.0^{f}	90.3^{f}	57.6^{e}	66.2^{e}
9	66.8^{e}	88.1^{f}	" ^{f}	65.5^{e}	68.1^{e}
10	73.1^{e}	90.7^{f}	90.9^{f}	73.9^{e}	69.5^{e}
11	79.4^{efh}	93.3^{fg}	" ^{hg}	79.1^{eh}	71.8^{e}
12	80.0^{efgh}	" ^{f}	91.5^{fh}	80.7^{ef}	77.0^{eg}
13	82.3^{e}	93.8^{e}	92.6^{e}	83.3^{e}	84.0^{e}
14	84.6^{e}	94.3^{e}	" ^{e}	84.9^{e}	87.3^{e}
15	85.7^{e}	" ^{e}	93.7^{e}	85.4^{e}	90.6^{e}
16	88.6^{e}	94.8^{e}	94.3^{e}	85.9^{e}	92.0^{e}
17	89.7^{e}	95.3^{e}	96.5^{e}	87.5^{e}	92.9^{e}
18	91.4^{e}	95.8^{e}	" ^{e}	90.6^{e}	" ^{e}
19	93.1^{e}	" ^{e}	" ^{e}	92.7^{e}	" ^{e}
20	94.8^{e}	" ^{e}	" ^{e}	95.3^{e}	" ^{e}
21	96.5^{e}	" ^{e}	" ^{e}	95.8^{e}	93.4^{e}
22	" ^{e}	98.9^{e}	97.6^{e}	96.9^{e}	96.7^{e}
23	" ^{e}	" ^{e}	98.2^{e}	97.4^{e}	98.1^{e}
24	-	-	-	-	-
25	-	-	-	-	-
26	-	-	-	-	-
27	98.2^{e}	99.4^{e}	98.8^{e}	99.0^{e}	99.5^{e}
28	99.3^{e}	" ^{e}	99.9^{e}	" ^{e}	" ^{e}
29	99.9^{e}	" ^{e}	" ^{e}	99.5^{e}	" ^{e}
30	" ^{e}	99.9^{e}	" ^{e}	" ^{e}	100.0^{e}
31	" ^{e}	" ^{e}	" ^{e}	" ^{e}	" ^{e}
CRG	13.25	19.98	17.09	13.24	13.73
n	175	194	181	191	213

*Treatment-days within a row sharing the same letter do not differ significantly ($P \lesssim .05$) (modified Smirnov Test).

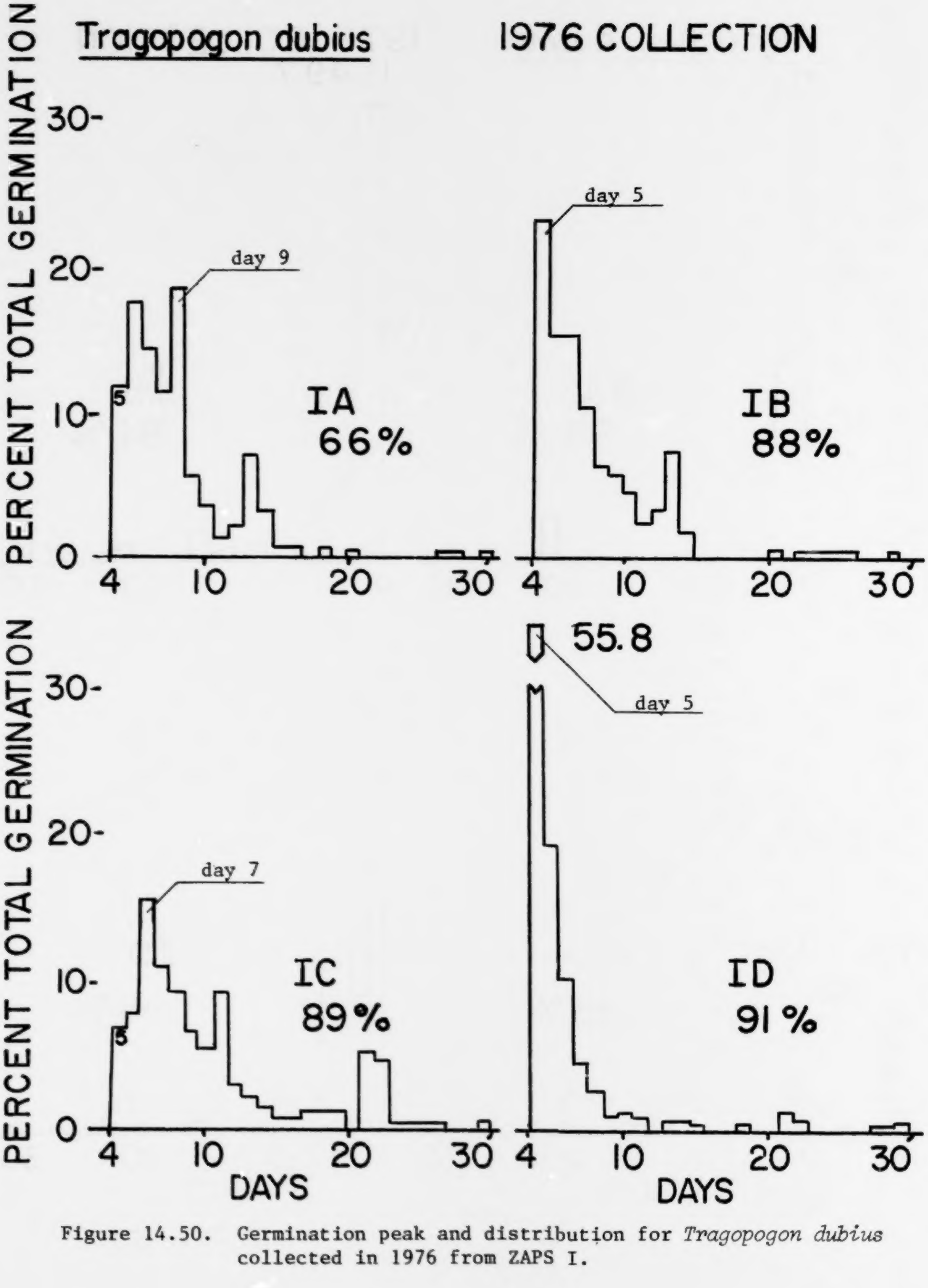


Figure 14.50. Germination peak and distribution for *Tragopogon dubius* collected in 1976 from ZAPS I.

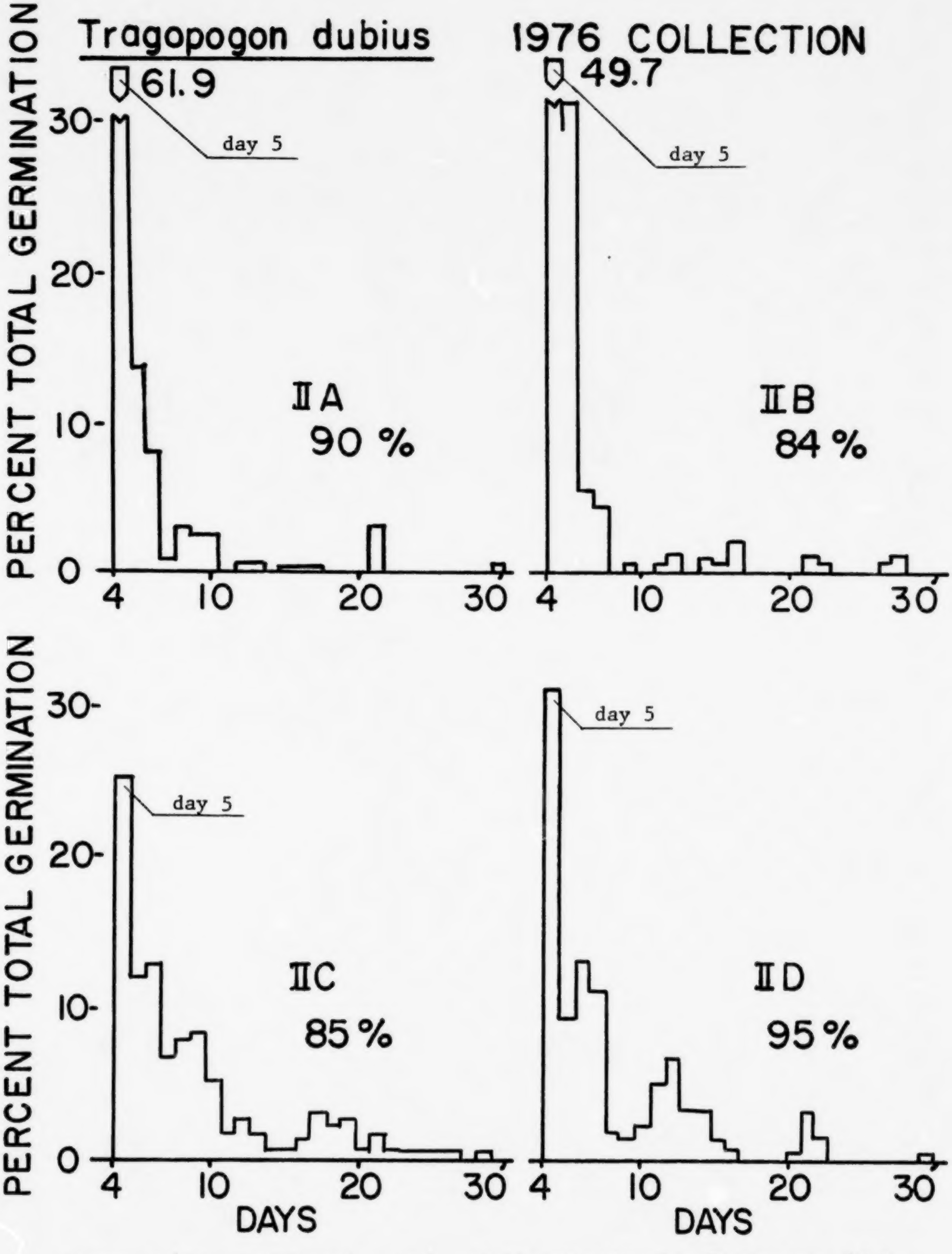


Figure 14.51. Germination peak and distribution for *Tragopogon dubius* collected in 1976 from ZAPS II.

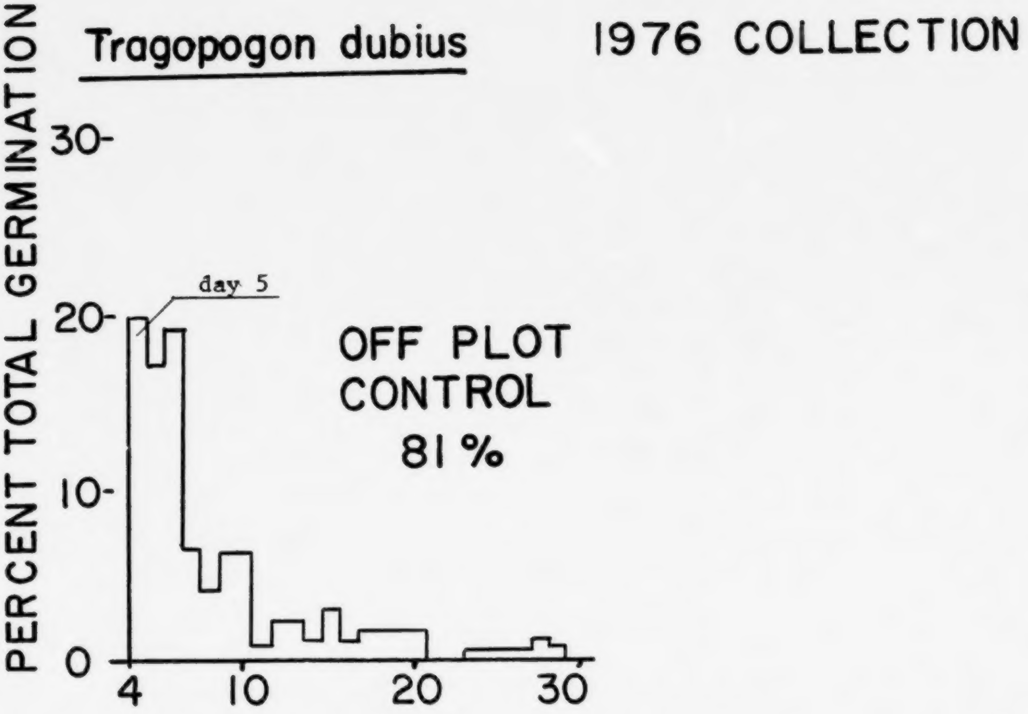


Figure 14.52. Germination peak and distribution for *Tragopogon dubius* collected in 1976 from the Off Plot Control.

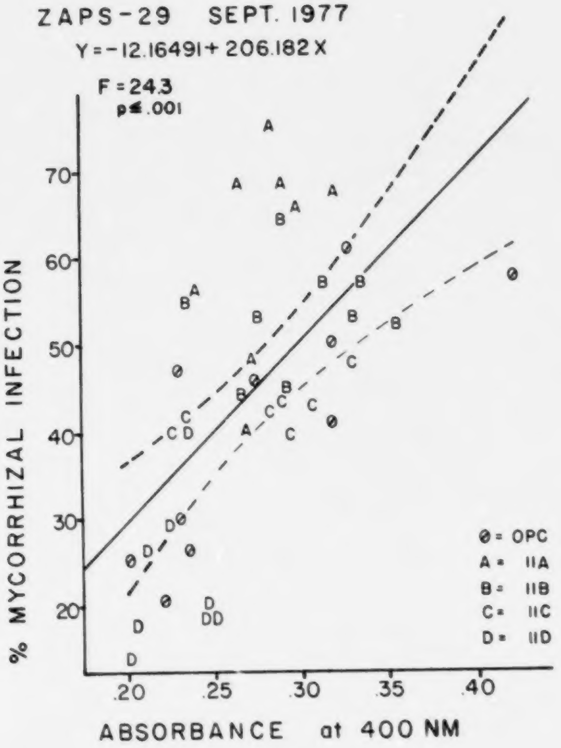


Figure 14.53. Regression of percent mycorrhizal infection on absorbance from collection 29 (mid-September) during 1977 on ZAPS II.

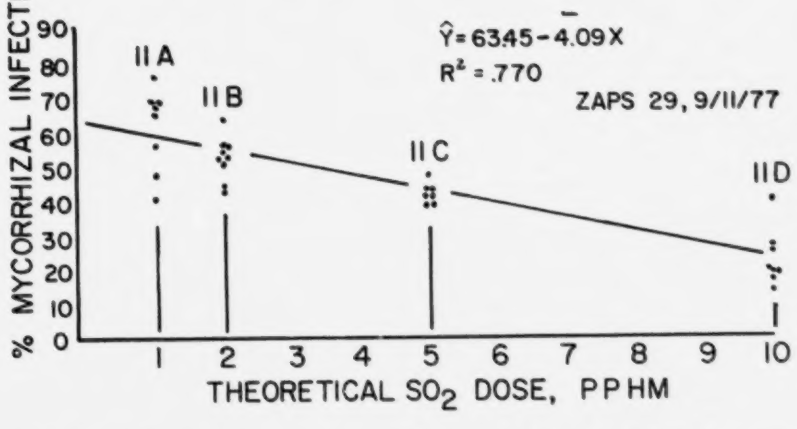


Figure 14.54. Regression of percent mycorrhizal infection on theoretical SO_2 dosage from collection 29 (mid-September) during 1977 on ZAPS II.

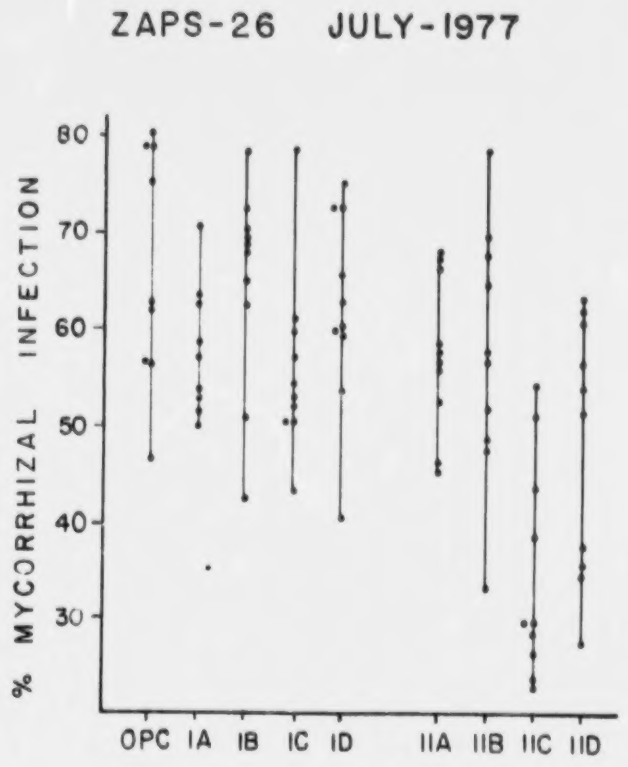


Figure 14.55. Percent mycorrhizal infection on ZAPS I and II from collection 26 (July) during 1977.

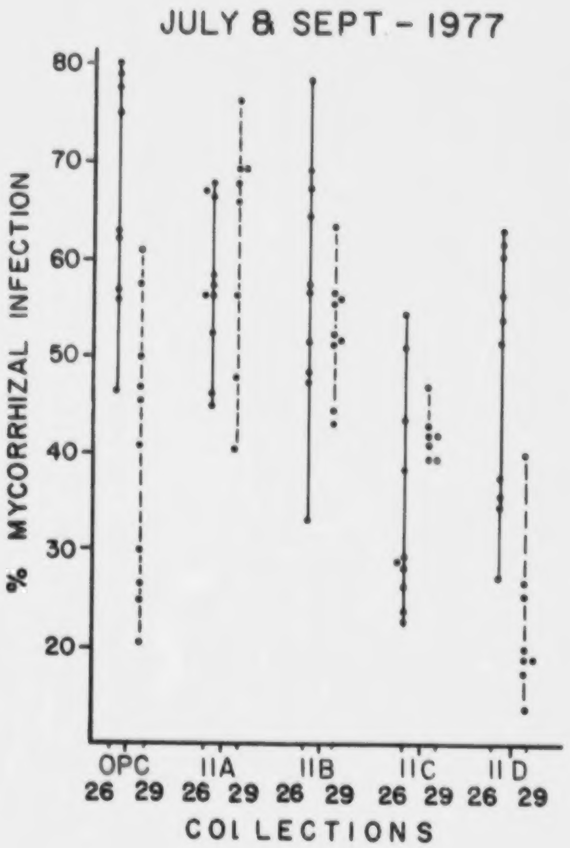


Figure 14.56. Percent mycorrhizal infection on ZAPS II from collections 26 and 29 (July and September) during 1977.

The data representing the mycorrhizal infection levels, as determined by Newman's point intersection technique as well as the corresponding absorbance readings, can be found in Appendix 14.4. The Kruskall-Wallis test indicated significant ($P < .001$) differences for the visually determined infection levels in both collection 26 ($H = 31.384$, $k = 8$) and collection 29 ($H = 26.314$, $k = 4$).

Specific plot pairs were examined for significant ($P \leq .05$) differences by the Wilcoxon two-sample test. Assuming no change in infection levels (H_0: $\mu_1 = \mu_2$), two alternative hypotheses were made:

(1) H_1: $\mu_1 > \mu_2$. Mycorrhizal populations decrease as the dosage delivered to the plot increases (for collection 26, when mycorrhizal data is available for both sites, a ZAPS II plot is considered to have received a higher canopy level concentation [see Section 10, Figure 10.4] than the "same" ZAPS I plot, so that infection levels for ZAPS I plot X > ZAPS II plot X).

(2) H_2: $\mu_1 < \mu_2$. Mycorrhizal infection levels are stimulated to increase as the dosage of SO_2 increases.

For collection 26, under the first alternative hypothesis (Table 14.19a), it can be seen that the infection level is depressed on the higher treatment plots. This pattern is particularly strong on IC, IIC, and IID, in contrast to the OPC and IB and to a lesser degree for IA, IIA, and IIB. Consistent with this alternative, IC and ID show higher infections than the similarly designated ZAPS II plots. Examining the second alternative hypothesis (Table 14.19b), the mid-July collection 26 also reveals significant ($P \leq .05$) differences that could be associated with a SO_2 stimulation effect. The B plots are higher than the A plots, but the pattern is broken by ID, and the limited number of significant cases (4 out of 36) can be ascribed in part to the expected number of Type 1 errors involved in multiple pair comparisons. For the fall collection 29, under the first alternative hypothesis (Table 14.20a), the mycorrhizal levels are strongly depressed, with the increasing ambient SO_2 concentrations on the ZAPS II site itself. Examining the second alternative hypothesis (Table 14.20b), the IIA and IIB plot infection levels are elevated, in contrast to the OPC.

Of interest was the significant ($P \leq .05$) discovery that percent mycorrhizal infection levels were negatively correlated (Spearman's rho = -0.238, n = 88) with total sulfur accumulated in leaves on the OPC and ZAPS I and II from collection 26. Although a positive correlation was found between sulfur in leaves and sulfur in roots ($r_s = 0.301$, $n = 88$) from the same collection, a significant ($P \leq .05$) correlation ($r_s = -0.088$, $n = 88$) was not found between percent mycorrhizal infection and total sulfur in the roots. A similar analysis of collection 29, which was comprised of ZAPS II and the OPC (ZAPS I was not scored for mycorrhiza), demonstrated a continuance of these relationships into the fall. The mycorrhizal infection decreased in individual plants as the sulfur content of the leaves increased ($r_s = -0.602$, $n = 42$). Root sulfur was correlated ($r_s = 0.312$, $n = 4$) with leaf sulfur, but sulfur in the roots did not appear to be directly ($\alpha = .05$) associated with a change in infection levels ($r_s = -0.051$, $n = 41$).

TABLE 14.19a. WILCOXON TWO-SAMPLE TEST FOR DIFFERENCES IN PERCENT MYCORRHIZAL INFECTION ON ZAPS I AND ZAPS II FROM COLLECTION 26 DURING MID-JULY, 1977, UNDER H_1

Percent Infection	IIC 35	IID 49	IC 56	IIA 58	IA 58	IIB 58	ID 62	IB 65
OPC 66	31*	17*	10*	8	8	8	4	1
IB 65	30*	16*	9*	7	7	7	3	
ID 62	27	13*	6	4	4	4		
IIB 58	23*	9	2	0	0			
IA 58	23*	9	2	0				
IIA 58	23*	9	2					
IC 56	21*	7						
IID 49	14							

H_0: $\mu_1 = \mu_2$

H_1: $\mu_1 > \mu_2$

i.e.: The plot (μ_1) receiving the lower SO_2 dosage will have higher infection levels.

*$P \leq .05$

TABLE 14.19b. WILCOXON TWO-SAMPLE TEST FOR DIFFERENCES IN PERCENT MYCORRHIZAL INFECTION ON ZAPS I AND ZAPS II FROM COLLECTION 26 DURING MID-JULY, 1977, UNDER H_2

Percent Infection	IIC 35	IID 49	IC 56	IIA 58	IA 58	IIB 58	ID 62	IB 65
OPC 66	31	17	10	8	8	8	4	1
IB 65	30	16	9	7*	7	7	3	
ID 62	27*	13	6*	4	4	4		
IIB 58	23	9	2	0	0			
IA 58	23	9	2	0				
IIA 58	23	9	2					
IC 56	21	7						
IID 49	14*							

H_0: $\mu_1 = \mu_2$

H_2: $\mu_1 < \mu_2$

i.e.: Increased SO_2 dosage on a plot (μ_2) will increase infection levels.

*$P \leq .05$

TABLE 14.20a. WILCOXON TWO-SAMPLE TEST FOR DIFFERENCES IN PERCENT MYCORRHIZAL INFECTION ON ZAPS II AND THE OPC FROM COLLECTION 29 DURING SEPTEMBER, 1977, UNDER H_1

Percent Infection	IID 23	OPC 41	IIC 42	IIB 53
IIA 62	39*	21	20*	9*
IIB 53	30*	12	11*	
IIC 42	19*	1		
OPC 41	18*			

H_0: $\mu_1 = \mu_2$
H_1: $\mu_1 > \mu_2$

i.e.: The plot (μ_1) receiving the lower SO_2 dosage will have higher infection levels.

*P $\leq$ .05

TABLE 14.20b. WILCOXON TWO-SAMPLE TEST FOR DIFFERENCES IN PERCENT MYCORRHIZAL INFECTION ON ZAPS II AND THE OPC FROM COLLECTION 29 DURING SEPTEMBER, 1977, UNDER H_2

Percent Infection	IID 23	OPC 41	IIC 42	IIB 53
IIA 62	39	21*	20	9
IIB 53	30	12*	11	
IIC 42	19	1		
OPC 41	18			

H_0: $\mu_1 = \mu_2$
H_2: $\mu_1 < \mu_2$

i.e.: Increased SO_2 dosage on a plot (μ_2) will increase infection levels.

*P $\leq$ .05

The morphology of both the mycorrhiza and the *A. smithii* roots were studied microscopically and a series of photomicrographs prepared (Figures 14.57 and 14.58). Highly swollen and abnormally large interior vesicles were found in roots from the IB plot, collection 26 (Figure 14.58G). Samples from IIB, collections 26 and 29, contained an extremely large number of exterior vesicles and attached hyphae (Figure 14.57C) compared to roots from any of the other plots sampled, particularly IID, collection 29, where no exterior vesicles could be found. A general reduction in root width and an increased browning were observed to be associated with increased SO_2 dosage on the ZAPS II collection 26. ZAPS I collection 26 and ZAPS I and II collection 29 were not examined for such characteristics.

DISCUSSION

In 1975, we hypothesized that the plant species on the medium and high treatment plots of the ZAPS sites would be severely damaged or killed outright during the growing season. In the TIR, we stated that this hypothesis had not proven valid after continuous fumigation during two growing seasons on ZAPS I and one growing season on ZAPS II (Gordon *et al.*, 1978). Studies carried out on these sites during the 1977 growing season demonstrated that damage to the plant species cannot be easily detected by field observation of necrotic foliage or measured quantitatively by loss of plant diversity or biomass (Dodd *et al.*, 1978). If the initial indications of damage in a grassland ecosystem cannot be ascertained either by acute visible leaf injury or through quantification of loss of diversity and/or net productivity after two or three years of continuous SO_2 fumigation at levels exceeding the average annual SO_2 concentrations in Anaconda, Montana (Montana DHES, 1978), or Sudbury, Ontario (McGovern and Balsillie, 1974; Linzon, 1973), the research efforts on the ZAPS sites have created even more uncertainty about the phytotoxicity of SO_2.

For instance, why do the aerial infrared photographs taken of the ZAPS sites in 1975, 1976, and 1977 (Taylor, 1976; Schott *et al.*, 1976; Osberg, 1978) visually demonstrate a definite gradation of reflectance across the different treatment plots on each of the ZAPS sites during and after the growing season, and yet no significant SO_2-caused necrotic or chlorotic spotting or flecking has been reported to date? We have observed significant amounts of early senescence of western wheat-grass foliage on the higher treatment plots (Gordon *et al.*, 1978), as had Dodd *et al.* (1978), but no one has reported any typical SO_2-caused foliar necrosis on the treatment plots. Therefore, any serious vegetation damage which may be occurring on the ZAPS sites is not being manifested as "typical" SO_2-caused foliar necrosis. If this is the case, field and controlled fumigation chamber research studies, which primarily depend upon quantification of the amount of SO_2-caused necrotic foliage to ascertain the dose-duration-response of plant species (Hill *et al.*, 1974; Davis *et al.*, 1966), are basically academic exercises which can form no basis for truly protective ambient SO_2 standards.

The ZAPS field investigation is a unique study and represents the first attempt to fumigate relatively large areas of a grassland ecosystem during several growing seasons. Because it is a unique experiment, great care must be taken to interpret the fumigations and their various parameters. One of

Figure 14.57. Endomycorrhizae of *Agropyron smithii*.

A. Exterior vesicles and hyphae from ZAPS plot IIB, collection 26.

B. Cross-section of root from the OPC, collection 29, showing interior vesicles in outer cortical cells.

C. Mass of exterior vesicles and hyphae from ZAPS plot IIB, collection 26.

D. Interior vesicles in outer cortex as viewed through epidermis; whole mount of root from OPC, collection 26.

Figure 14.58. Endomycorrhizae of *Agropyron smithii*.

E. Arbuscular stage in whole mount of root from ZAPS plot IIB, collection 26.

F. Cross-section of root from OPC, collection 29, showing hyphae entering through root hair.

G. Enlarged, abnormal interior vesicles in root from ZAPS plot IB, collection 26.

H. Interior vesicles and hyphae in whole mount of root from ZAPS plot IIB, collection 26.

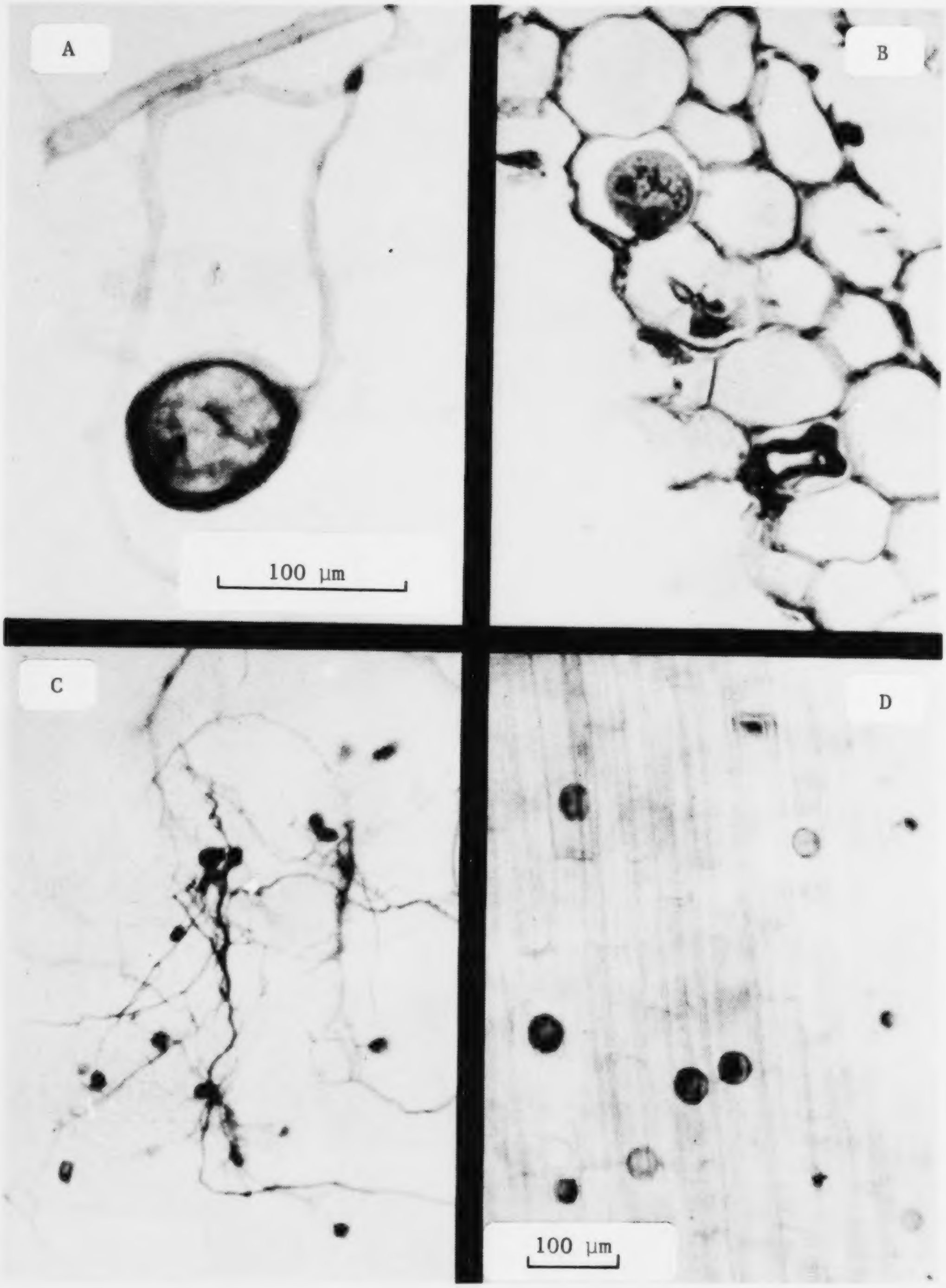

A
B
C
D
100 µm
100 µm

Figure 14.57. Endomycorrhizae of *Agropyron smithii*.

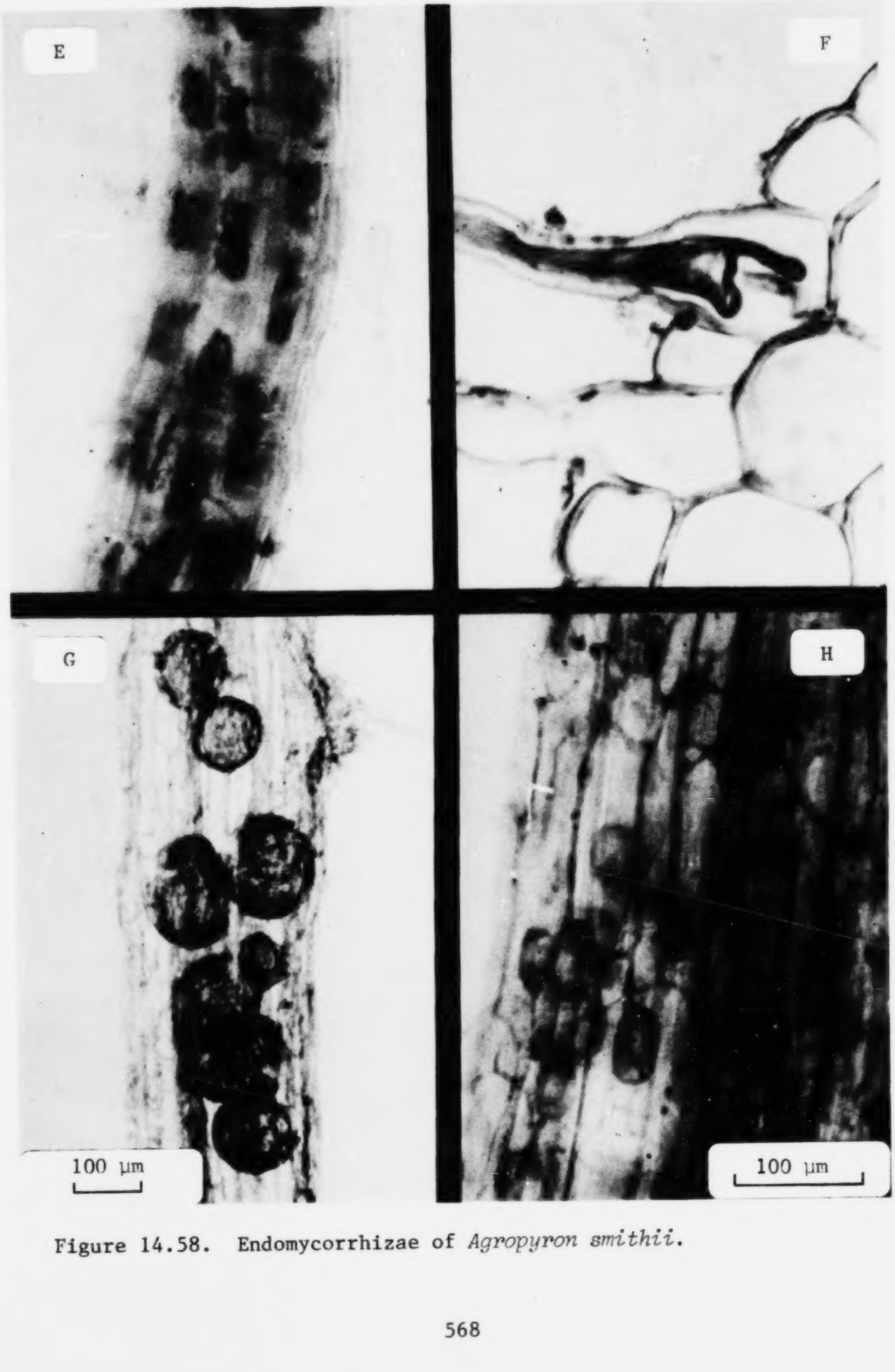

E
F
G
H
100 µm
100 µm

Figure 14.58. Endomycorrhizae of *Agropyron smithii*.

the major problems on the ZAPS sites has been to determine the actual amount of SO_2 impacting the flora and fauna on treatment plots. Ambient concentrations determined by the continuous analyzer are significantly higher (Section 10) than the concentrations maintained within the plant canopy on the intentionally-fumigated plots, reflecting the location of the sampling probes at the extreme top of the canopy. The interrelationship of dose and species response cannot be fully described at this time, as the available ambient data only describe an upper limit of the SO_2 concentrations. Until the vertical profile of SO_2 concentrations is quantified, this limitation of the ZAPS work to setting protective ambient air standards for grasslands must be recognized.

Another major problem that became evident during the three seasons of fumigation was the fact that the A plots, or controls, on both ZAPS sites were receiving quantifiable levels of SO_2. Thus there are, in actuality, no real control plots at the ZAPS sites. While this is not an insurmountable problem, it is important to remember that the sulfur concentrations in the physiological responses of flora and fauna on the A plots cannot be used as zero dosage comparisons to species on the B, C, and D plots. In Section 5 of this report, Gordon *et al.* reported the problems encountered in the Trail, B.C., study (Katz *et al.*, 1939), because the investigators believed that a control area actually existed 70 to 90 miles downwind from a smelter. The ambient SO_2 concentrations in southeastern Montana before the coal-fired power plants at Colstrip began operations have been reported by several state and federal agencies, as well as other investigating teams (Department of Health and Environmental Sciences, Department of Natural Resources and Conservation, the Environmental Protection Agency, PEDCo-Environmental Specialists, Inc., the Northern Cheyenne Tribe, and Battelle Pacific Northwest Laboratories) The conclusion of all was that the average 24-hour ambient concentration of SO_2 was below detectable levels before the Colstrip power plants went on-line. The OPC data we utilized during 1976 and 1977, in juxtaposition with the data obtained from our ZAPS studies, are important in understanding the potential impact of SO_2 at the concentrations being received by the flora and fauna on the A treatment plots.

The last major problem encountered during the four years of ZAPS studies is the real possibility that our study designs, which evolved before and after the ZAPS investigations, are potentially incapable of quantifying the long-term impacts of ambient SO_2 at any realistic annual concentration (0.5 to 1.0 pphm) when applied to a short-term fumigation study (4 years vs. 30 years). These concentrations could and probably will occur during the next 40 years if the Fort Union Basin becomes industrialized because of its vast coal deposits. Our primary concern is that potential long-term consequences of chronic air pollution to the grassland and ponderosa pine ecosystems cannot be easily or quickly ascertained. For instance, until the viability and germination studies on the 1976 seed crop of several species from the ZAPS sites were completed, analyzed, and studied during 1977, we did not realize that: (1) There was an impact on these processes by SO_2, and (2) this type of impact study has rarely been utilized in field or controlled fumigation studies. Also, our work on the mycorrhizal association of western wheatgrass during 1977 and the winter of 1978 disclosed that there is a disruption and/or change in this association in vegetation fumigated with different levels of SO_2. It has not been determined whether this disruption and/or change is caused directly or indirectly by the SO_2 fumigation of host species, but it is known and fairly well accepted that this reciprocal symbiotic relationship between fungi and root tissues probably occurs in all higher plant species and is beneficial to their health and growth (Zak, 1964). The mycorrhizal association of the other numerous grass and forb species on the ZAPS sites has neither been identified nor studied. If the effects of SO_2 on possible mycorrhizal associations of these other species are to be determined, a study period of several years would be required.

This unique field SO_2 fumigation study was initiated to better understand the potential impact threshold levels of ambient SO_2 upon a grassland ecosystem. Thus far, the ZAPS study has demonstrated that these impacts are probably more subtle and evolve more slowly than any of the investigators originally believed. If there is a quantifiable ambient SO_2 threshold, below which no important biological and/or economic damage will occur, it has not been established at this time.

Sulfur accumulation in the foliage of the grass and forb species collected at the ZAPS sites during 1976 demonstrated that vegetation analyses can be utilized in pristine areas to determine the presence of chronic levels of SO_2. The limitations of this technique, however, may become apparent after two or three years of continuous, chronic fumigation because it appears that residual sulfur in the root tissues or biologically available in the soil solutions may mask much of the ambient sulfur taken in by foliage during any single year at chronic SO_2 levels (0.5 to 2 pphm). Cronan *et al.* (1978) have documented the large contribution (76%) of sulfate anion to the electrical charge balance in the soil water solution of chronically-polluted ecosystems of New Hampshire. Sulfur levels in western wheatgrass roots collected at the two Anaconda sites during 1977 at three different periods during the growing season adequately demonstrate the presence of residual sulfur in these plant parts in chronically- and acutely-polluted areas.

Probably the most important aspects of sulfur accumulation studies on the ZAPS studies thus far are that:

(1) Sulfur accumulation is a continuous phenomenon in the foliage of most species on the ZAPS being tested. If this continuous increase in live foliage of the plant sample cannot be detected, the analysis of current year's dead tissue will more effectively demonstrate the phenomenon.

(2) Sulfur accumulation in foliage collected from the ZAPS sites can be used to distinguish the higher SO_2 dosage areas from lower dosage areas within each plot (see Tables 14.3 and 14.4).

(3) Plants with higher sulfur levels in foliage across treatment plots generally manifest earlier leaf senescence and greater reductions in seed viability, percent seed germination, speed of germination, and mycorrhizal association. Tests for significant correlations between these quantifiable changes and the sulfur levels in the foliage have been undertaken only for mycorrhizal associations at

this time. Of the grass and forb species collected during the 1977 season, only western wheatgrass has been analyzed for sulfur content. Thus we believed it too early to test for other possible correlations prior to finishing all studies on the 1977 foliage and seed collections.

Simulated "rainfall event" and "overwintering" experiments on foliage from the D plot demonstrate that a portion of the sulfur on and possibly in the foliage from the ZAPS sites can be removed. Because sulfur can be reduced in such a manner, the use of such accumulation in foliage to determine chronic ambient SO_2 fumigations will probably have to take into consideration the amounts and durations of rainfall in the study area.

In the TIR we reported that species of grasses and forbs common on the ZAPS sites would be grown on Anaconda soils brought to the Botany gardens in Missoula to determine the specific contribution of the soil to residual sulfur levels. These species were established on soil collected from the DHES Highway Junction and Pumphouse air monitoring sites in Anaconda. Data on the influence of 80 years of SO_2 pollution on these soils in relationship to potential excessive sulfur uptake is being gathered and will be reported in the Fifth Interim Report.

Analysis of western wheatgrass samples collected during 1977 from the two Anaconda air monitoring sites (Table 14.6) show that after years of low and high level SO_2 fumigation, the sulfur content of root tissue exceeds that accumulated by the foliage during the growing season. Portions of the excess sulfur undoubtedly end up in the rhizosphere of SO_2-impacted plants. Unfortunately, very few air pollution studies have included research to ascertain the potential indirect impacts of sulfur accumulation in the soils on the microflora and microfauna.

Lack of time during 1977 allowed only a limited preliminary study on the population or presence of mycorrhizal association in the root tissues of the Anaconda samples. This study disclosed that mycorrhizal fungi were not present in root tissues from the DHES Highway Junction site. This cannot be attributed singularly to the SO_2 or sulfur levels in the ambient air or plant tissues because areas of the Deerlodge Valley affected by the Anaconda copper smelter emissions also are seriously impacted by heavy metals which have eliminated a large portion of the soil fungal populations (Hartman, 1975).

The ZAPS sites, however, unlike the Anaconda area, are being impacted by only one phytotoxic gas, and studies on the potential impacts of any excessive rhizosphere sulfur levels should be explored. The indirect effects of SO_2 fumigation upon ecosystems may be the most serious and long-lasting impact caused by the emissions of coal-fired power plants, which release relatively small amounts of toxic trace metals in comparison to nonferrous smelters.

The germination and viability studies carried out in 1976 on seed collections from the ZAPS sites were essentially a pioneering effort to determine the impacts of SO_2 fumigation upon ecosystems. While earlier air pollution investigations (Katz *et al.*, 1939) have included viability tests on seeds from SO_2-impacted trees, we know of no investigation in which seeds from plants fumigated with known and different concentrations of SO_2 were tested for viability, percent germination, and rate of germination. Because the 1976 seed collections were our first attempt to ascertain the potential impacts of different SO_2 levels at the ZAPS sites on seed viability and percent germination, it is probably premature for us to discuss the possible consequences of seed damage from SO_2 fumigation until we have completed these same studies on the 1977 seed collections.

However, there are three assumptions which are suggested at this time by the results of these seed studies. First, there have been numerous articles in the air pollution literature discussing the possible existence of invisible injury to plants caused by phytotoxic gases. If one accepts that visible injury is damage to any portion of the plant structure which can be determined by careful visual examination or by weighing or counting the plant structures, seed viability and percent germination rate affected by phytotoxic gases should be considered an invisible injury. The numerous investigators who have fumigated various plant species with various levels of SO_2 to determine the effects on anatomical structure or metabolic pathways of the plant species have not tested the seed viability and percent germination of these species. Any damage remained invisible to the investigators and was not reported in the literature.

The second point is the reduction in seed weight across the treatment plots which, in general, paralleled the reduction observed in viability, percent germination, and rate of germination. This loss of seed weight in the grass species has some serious implications, not only to these species but very possibly to the yields of agricultural grain species grown throughout the Fort Union Basin.

The third point deals with the implications of the results obtained from the germination studies of the seeds of *Tragopogon dubius*, an introduced species to the Fort Union Basin and an unwanted forb. The data obtained thus far from the seed germination studies on *T. dubius* suggest that SO_2 fumigations may indirectly enhance both percent germination and rate of germination. If this is true, the impacts of increased colonization of both grazing lands and reclaimed strip mined lands by this forb species could cause a loss of carrying capacity and a reduction in the success of reclamation efforts in southeastern Montana.

A normal rate of seed viability of the indigenous plant species of this grassland ecosystem of southeastern Montana is an extremely important part of the continuing health and growth of this agricultural area. Long-term impacts could be serious if SO_2 fumigations at low chronic or high acute concentrations reduce seed viability or change the rate of germination by extending the time needed for seeds to germinate and establish their roots in the soils of this semi-arid area. Because of the importance of seed viability and the results thus far obtained in tests on our 1976 seed collections, we have continued these studies on 1977 seeds and will also be collecting them during 1978 for future studies.

We conducted vegetative phenology studies of western wheatgrass during the past two growing season (1976-77). During 1977, we expanded the number of tagged plants by increasing the number of subplots used in the study. It is important to understand the rate of leaf senescence across the treatment plots of the two ZAPS sites. This became apparent when we realized that senescence is the only clear visible response of western wheatgrass to SO_2 dosages, not the necrotic leaf lesions or the tan/ivory chlorosis obtained in controlled, acute fumigation chamber studies (Tingey *et al.*, 1978; Hill *et al.*, 1974). Early leaf senescence is the visible manifestation of this grass species to chronic levels of SO_2 fumigation and is quantified only after careful comparison of senescence rates on different treatment plots over the entire growing season. Therefore, it appears that the data in the literature on the impacts of short-term, acute SO_2 fumigations are of relatively little use in preparing or establishing ambient SO_2 standards.

Data on the rate of leaf senescence (or percent necrotic) of western wheatgrass (Tables 14.10a and 14.10b) show that while there are significant differences between some of the treatment plots on both ZAPS sites, these are not necessarily related to each dosage level. There was never more than 15% difference in the number of necrotic leaves between any of the treatment plots, including the OPC, at any given study period. If leaf necrosis is the criteria used for damage on the ZAPS sites, it seems that either there are few or no consequential impacts from the chronic SO_2 fumigations, or leaf necrosis is not an adequate indication of air pollution impacts on grassland ecosystems. In Section 5, Gordon *et al.* discuss the fact that air pollution damage to pine foliage can mimic those normal damage symptoms which occur on foliage collected from pristine areas. This discussion of data on conifer foliage pathologies suggests that no single damage manifestation can be utilized to determine the differences between health/growth/disease characteristics of foliage collected from chronically-polluted and pristine areas. If one compares the percent necrotic leaves of western wheatgrass from the OPC to those from the ZAPS sites (Tables 14.10a and 14.10b), one should understand that if an entire western wheatgrass ecosystem was exposed to ambient air SO_2 levels of 1 to 5 pphm for varying durations during the growing season, field investigators would not be able to detect the potential impacts by visually inspecting the foliage.

Although by no means conclusive, the results of this investigation indicate that certain levels of SO_2 fumigation, such as those on the ZAPS C and D plots, have a definite detrimental impact on mycorrhizal populations which inhabit *Agropyron smithii*. Besides data generated from the 1977 collection period, photographed morphological evidence substantiates the finding of impairment of the symbiotic state by SO_2 (Figures 14.57 and 14.58).

Statistically significant were the findings that: (1) Spectrophotometer absorbance readings are related to and potentially predictive of percent mycorrhizal infection; (2) mycorrhizal levels were negatively correlated with sulfur concentrations in the leaves of *A. smithii* from the same collection period; (3) mycorrhizal levels were not correlated with sulfur levels in roots, and (4) theoretical SO_2 dosage was inversely related to mycorrhizal infection levels on ZAPS II in the fall collection.

Taking into considerations heterogeneity of biological response within naturally-varying populations, several additional trends or patterns emerged from this study. For example, roots from C and D plots consisted of a greater number of thinner, senescent roots, whereas roots from the OPC tended to be thicker, less senescent, and with a fatter and possibly more persistent cortex. The following high probability trends or patterns also emerged from this study: (1) High levels of infection and exterior vesicle formation were associated with low SO_2 dosage plots; (2) populations generally declined or fewer roots were infected on the higher (C and D) dosage plots; (3) abnormal, swollen interior vesicles, similar to those formed as a result of a lack of nitrogen, were found on the IB plot, and (4) a trend towards less variability of infection levels was associated with increasing fumigation levels.

The fact that EDM exist within an enormous variety of plant species and flourish under an extremely wide range of environmental conditions suggests that these are organisms of widely adaptable behavior and tolerance. The depressions in their population seen on the ZAPS plots, which are edaphically far more similar than dissimilar, are thus most likely an indirect result of unusual modifications in the health state of the host plant, rather than a direct result of SO_2 fumigation. Explanations of the manner in which mycorrhizae are impacted by alterations in host plant metabolism are a matter worthy of consideration, further experimentation, and future investigation. At this point in time, explanations of this sort would be highly speculative. However, by utilizing information garnered from SO_2 impact studies on other obligate parasites or symbiotic states, one can obtain a greater understanding of the trends elicited in this study.

Occurrence of an unusually large number of exterior vesicles on roots from IIB, when compared to any of the other plots, might be interpreted in the following manner: Either the fungus has entered into its sporulating stage as part of a repetitive infectious process due to the very high suitability of the habitat, or the fungus is undergoing a typical reaction to harsh, extreme, or otherwise adverse environmental conditions. This and observations of very swollen interior vesicles on the IB plot lead to the conclusion that the habitat is quite unsuitable. Although such a change could perhaps also be wrought by stress induced as a consequence of sudden alterations in the host plant's competitive ability, the condition is, nevertheless, abnormal.

With respect to the impact of SO_2 on other aspects of plant metabolism, Dodd *et al.* (see Section 13) noted a tendency towards a decrease in rhizome biomass across the plots, which coincides with the experimenter's observation of a similar decrease in root size. A surplus of carbohydrates in roots is thought by some to be the main fungal attractant, utilization of such by EDM preventing the attraction of more serious pathogens. If SO_2 fumigation caused either a decrease in proper photoassimilates transported to the roots due to an overall decrease in root carbohydrates, or an increase of tissue leakage within the roots, suitable mycorrhizal nutritional requirements might not be maintained.

A well-established theory of plant pathogenicity associates tissue leakage or quantities of cellular materials released by exosmosis with disease-induced stress (Wheeler and Hanchey, 1968). That mycorrhizae do not increase tissue leakage is evidenced by their induction within a host of increased cortical and cytoplasmic volume, increased mineral uptake, and an overall increase in vigor. Plasmolysis and consequent tissue leakage, however, are characteristic of SO_2 damage (Ziegler, 1975). Leakage of vascular cylinder contents to root exteriors would most likely be deleterious to EDM because it is within the phloem and endodermis that phenols and potentially fungitoxic quinones exist in greatest concentration. It is thought that only in the more successful pathogens, those which cause a systemic infection or otherwise penetrate the endodermis and enter the vascular cylinder, does there exist the capability of oxidizing the phenols and quinones to non-toxic insoluble pigments (Miles, 1968). Less successful pathogens are considered incapable of extensive quinone oxidation. Although the relationship between such fungitoxic phenols and EDM is unknown, the fact that EDM do not penetrate the endodermis and vascular cylinder is suggestive of a chemical barrier within these tissues, which would be deleterious to EDM if it leaked, as might happen under conditions of unusual stress.

Once again, it must be stated that the physiological relationship, in terms of metabolic pathways, has been virtually unexplored in the case of EDM, due to the lack of technique for culturing the endophyte separately from the host. In view of the delicate metabolic balance exhibited in any symbiotic state and the potential for disruption of this state in EDM by SO_2, theories concerning such at this point in time, however speculative, are nonetheless pertinent.

In conclusion, it is suggested that in response to SO_2 fumigation, the root system of *Agropyron smithii* on the ZAPS sites is undergoing modification resulting in either exclusion or lessening of the mycorrhizal habit.

CONCLUSIONS

The 1977 studies on vegetation collected on and/or studied in situ at the ZAPS sites demonstrate that there are significant impacts to selected species fumigated by various SO_2 concentrations. Levels of SO_2 between and within treatment plots are variable and, in general, substantially lower in the vicinity of most of the foliage of the plant species than reported in earlier publications. The studies conducted and completed during 1976 and 1977 indicate that seed viability and germination studies across treatment plots and the continuation and expansion of mycorrhizal association studies of western wheatgrass would be the most productive investigations during the 1978 season. Both of these studies are unique to controlled fumigation, and grass mycorrhizal studies have never previously been reported in field investigations of air pollution impacts.

Our 1978 studies will attempt to verify the results obtained in these two studies during the last interim report period. To adequately test the results, we will increase the mycorrhizal collections from each treatment plot and utilize collections from earlier portions of the growing season (June) as well as at the end of the seasons. Also, another OPC will be established in the Fort Howes district of the Custer National Forest during the spring of 1978 so that adequate sample sizes can be utilized to contrast collections from the ZAPS treatment plots.

From the studies completed during the last three years on the ZAPS sites, it has become totally evident that seasonal low-level SO_2 fumigation impacts upon grassland ecosystems are subtle and difficult or impossible to quantify by examining foliage for SO_2-caused necrotic lesions or typical discoloration. It also has become apparent that SO_2 causes impacts directly or indirectly to plant functions (seeds and mycorrhizal association) which have remained invisible to both field and fumigation chamber investigators.

REFERENCES

Ainsworth, G.C., and A.S. Sussman, eds. 1965. The Fungi: Volume I. The Fungal Cell. Academic Press, New York. 748 pp.

Ainsworth, G.C., and A.S. Sussman, eds. 1968. The Fungi: Volume III. The Fungal Population. Academic Press, New York. 738 pp.

Ashenden, T.W., and T.A. Mansfield. 1977. Influence of Wind Speed on the Sensitivity of Ryegrass to SO_2. J. Exp. Bot., 28(104):729-735.

Association of Official Seed Analysts. 1970. Rules for Testing Seeds, 1970. Proc. Assoc. Off. Seed Anal., 60(2):116.

Baylis, G.T.S. 1967. Experiments on the Ecological Significance of Phycomycetous Mycorrhizas. New Phytol., 66:231-243.

________. 1972a. Fungi, Phosphorous, and the Evolution of Root Systems. Search, 3(7):257-258.

________. 1972b. Minimum Levels of Available Phosphorous for Non-mycorrhizal Plants. Plant and Soil, 36:233-234.

________. 1974. The Evolutionary Significance of Phycomycetous Mycorrhizas. In: Mechanisms of Regulation of Plant Growth, R.L. Bieleski, A.R. Ferguson, and M.M. Cresswell, eds. Bulletin 12. The Royal Society of New Zealand, Wellington. pp. 191-193.

Bawden, F.C. 1957. The Role of Plant Hosts in Microbial Ecology. Symp. Soc. Gen. Microbiol., 7:299-314.

Becker, W.N., and J.W. Gerdemann. 1977. Colorimetric Quantification of Vesicular-Arbuscular Mycorrhizal Infection in Onion. New Phytol., 78:289-295.

Biddulph, O., S. Biddulph, R. Cory, and H. Koontz. 1958. Circulation Patterns for Phosphorous, Sulphur, and Calcium in the Bean Plant. Plant Physiol., 33:293-300.

Bird, G.W., J.R. Rich, and S.U. Glover. 1974. Increased Endomycorrhizae of Cotton Roots in Soil Treated with Nematicides. Phytopath., 64(1):48-51.

Bleasdale, J.K.A. 1973. Effects of Coal-Smoke Pollution Gases on the Growth of Ryegrass (*Lolium perenne* L.). Environ. Pollut., 5:275-285.

Booth, J.A., G.O. Thorneberry, and M. Lujan. 1976. Crop Reactions to Sulfur Dioxide in New Mexico. Bulletin 645. Agricultural Experiment Station, University of New Mexico, Las Cruces, New Mexico. 27 pp.

Butler, E.J. 1939. The Occurrence and Systematic Position of the Vesicular-Arbuscular Type of Mycorrhizal Fungi. Trans. Brit. Mycol. Soc., 22:274-301.

Cocking, W.D. 1973. Plant Community Damage and Repairability Following Sulfur Dioxide Stress on an Old-Field Ecosystem. Ph.D. Thesis, Rutgers University, New Brunswick, New Jersey. 133 pp.

Conover, W.J. 1971. Practical Nonparametric Statistics. John Wiley & Sons, Inc., New York. 462 pp.

Couey, H.M. 1965. Inhibition of Germination of *Alternaria* Spores by SO_2 Under Various Moisture Conditions. Phytopath., 55(5):525-527.

Cronan, C.S., W.A. Reiners, R.C. Reynolds, and G.E. Lang. 1978. Forest Floor Leaching: Contributions from Mineral, Organic, and Carbonic Acids in New Hampshire Subalpine Forests. Science, 200:309-311.

Cox, G., and F. Sanders. 1974. Ultrastructure of the Host-Fungus Interface in a V-A Mycorrhiza. New Phytol., 73:901-912.

________, and P.B. Tinker. 1976. Translocation and Transfer of Nutrients in Vesicular-Arbuscular Mycorrhizas. I. The Arbuscule and Phosphorous Transfer: A Quantitative Ultrastructural Study. New Phytol., 77:371-378.

Crush, J.R. 1974. Plant Growth Responses to VA Mycorrhiza. VII. Growth and Nodulation of Some Herbage Legumes. New Phytol., 73:745-754.

Daft, M.J., and A.A. El-Giahmi. 1974. Effect of *Endogone* Mycorrhiza on Plant Growth. VII. Influence of Infection on the Growth and Nodulation in French Bean (*Phaseolus vulgaris*). New Phytol., 73:1139-1347.

________, and T.H. Nicolson. 1966. Effect of *Endogone* mycorrhiza on Plant Growth. II. Influence of Soluble Phosphate on Endophyte and Host in Maize. New Phytol., 68:945-952.

________. 1969. Effect of *Endogone* Mycorrhiza on Plant Growth. III. Influence of Inoculum Concentration on Growth and Infection in Tomato. New Phytol., 68:953-961.

________, and B.O. Okusanya. 1973. Effect of *Endogone* Mycorrhiza on Plant Growth. VI. Influence of Infection on the Anatomy and Reproductive Development in Four Hosts. New Phytol., 72:1333-1339.

Davis, C.R., D.R. Howell, and G.W. Morgan. 1966. Sulphur Dioxide Fumigations of Range Grasses Native to Southeastern Arizona. J. Range Management, 19(2):60-64.

Dixon, J.W., and F.J. Massey, Jr. 1957. Introduction to Statistical Analysis. Second Edition. McGraw-Hill, New York. 488 pp.

Dodd, J.L., W.K. Lauenroth, R.K. Heitschmidt, and J.W. Leetham. 1978. First-Year Effects of Controlled Sulfur Dioxide Fumigation on a Mixed Grass Prairie Ecosystem. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Third Interim Report, Colstrip, Montana, December, 1977, E.M. Preston and R.A. Lewis, eds. EPA-600/3-78-021, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 345-375.

Duncan, D.B. 1955. Multiple Range and Multiple F Test. Biometrics, 11:1-41.

Eddleman, L.E. 1977a. Personal Communication. School of Forestry, University of Montana, Missoula, Montana.

________. 1977b. Indigenous Plants of Southeastern Montana. I. Viability and Suitability for Reclamation in the Fort Union Basin. Special Publication Four. Montana Forest and Conservation Experiment Station, School of Forestry, University of Montana, Missoula, Montana.

________, and P.S. Doescher. 1977. Selection of Native Plants for Spoils Revegetation Based on Regeneration of Characteristics and Successional Status. Completion Report under Contract No. 31-109-38-3632. Argonne National Laboratory, Argonne, Illinois.

Fisher, R.A. 1954. Statistical Methods for Research Workers. Twelfth Edition. Oliver & Boyd, Edinburgh. 356 pp.

Fitter, A.H. 1977. Influence of Mycorrhizal Infection on Competition for Phosphorous and Potassium by Two Grasses. New Phytol., 79:119-125.

Garrett, S.D. 1956. Biology of Root-Infecting Fungi. Cambridge University Press, New York. 293 pp.

Gerdemann, J.W. 1955. Wound-Healing of Hyphae in a Phycomycetous Mycorrhizal Fungus. Mycologia, 47:916-918.

________. 1964. The Effect of Mycorrhiza on the Growth of Maize. Mycologia, 56:342-349.

________. 1968. Vesicular-Arbuscular Mycorrhiza and Plant Growth. Ann. Rev. Phytopath., 6:397-418.

________, and J.M. Trappe. 1973. The Endogonaceae in the Pacific Northwest. Mycologia Memoir No. 5. The New York Botanical Garden, New York. 76 pp.

Gilmore, A.E. 1971. The Influence of Endotrophic Mycorrhizae on the Growth of Peach Seedlings. J. Am. Soc. Hort. Sci., 96:35-38.

Gordon, C.C., C.E. Carlson, and P.C. Tourangeau. 1976. A Cooperative Evaluation of Potential Air Pollution Injury and Damage to Coniferous Habitats on National Forest Lands Near Colstrip. Report No. 76-12.

USDA Forest Service, Northern Region, Missoula, Montana, and Environmental Studies Laboratory, University of Montana, Missoula, Montana. 89 pp.

________, P.C. Tourangeau, and P.M. Rice. 1978. Effects of Low-Level SO_2 Exposure on Sulfur Accumulation and Various Plant Life Responses of Some Major Grassland Species Under Field Conditions. In: The Bioenvironmental Impact of a Coal Fired Power Plant, Third Interim Report, Colstrip, Montana, December, 1977, E.M. Preston and R.A. Lewis, eds. EPA-600/3-78-021, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 399-472.

Gray, L.E., and J.W. Gerdemann. 1969. Uptake of Phosphorous-32 by Vesicular-Arbuscular Mycorrhizae. Plant and Soil, 30(3):415-422.

________. 1973. Uptake of Sulphur-35 by Vesicular-Arbuscular Mycorrhizae. Plant and Soil, 39:687-689.

Grubbs, F.E. 1969. Procedures for Detecting Outlying Observation in Samples. Technometrics, 11(1):1-21.

Grywacz, A. 1971. The Influence of Industrial Air Pollution on Pathological Fungi of Forest Trees. Sylvan, 155:55-67.

Hacskaylo, E. 1972. Mycorrhiza: The Ultimate in Reciprocal Parasitism. Bioscience, 22:577-583.

Harley, J.L. 1959. The Biology of Mycorrhiza. Interscience Publishers, Inc., New York. 233 pp.

Hartman, L.M. 1975. Fungal Flora of the Soil as Conditioned by Varying Concentrations of Heavy Metals. Ph.D. Thesis, University of Montana, Missoula, Montana. 147 pp.

Hayman, D.S., J.M. Barea, and R. Azcon. 1976. Vesicular-Arbuscular Mycorrhiza in Southern Spain; Its Distribution in Crops Growing in Soil of Different Fertility. Phytopathologia mediterranea, 15(1):1-6.

Heagle, A.S. 1973. Interactions Between Air Pollutants and Plant Parasites. Ann. Rev. Phytopath., 11:365-388.

Hibben, C.R. 1966. Sensitivity of Fungal Spores to Sulphur Dioxide and Ozone. Phytopath., 56:880. (abstract)

Hill, A.C., S. Hill, C. Lamb, and T.W. Barrett. 1974. Sensitivity of Native Desert Vegetation to SO_2 and to SO_2 and NO_2 Combined. J. Air Poll. Contr. Assoc., 24(2):153-157.

Hitchcock, C.L., and A. Cronquist. 1973. Flora of the Pacific Northwest. University of Washington Press, Seattle. 730 pp.

Hoover, M.M., *et al.* 1948. The Main Grasses for Farm and Home. In: Grass: The Yearbook of Agriculture. U.S. Government Printing Office, Washington, D.C. pp. 642-645.

Jackson, N.E., R.E. Franklin, and R.H. Miller. 1972. Effects of Vesicular-Arbuscular Mycorrhizae on Growth and Phosphorous Content of Three Agronomic Crops. Proc. Soil Sci. Soc. Am., 36:64-67.

Jorgensen, H.E. 1970. A Life History Study of *Agropyron smithii* Ryalb. in Central Montana with Related Effects of Selected Herbicide Treatment of Rangeland. Ecological Effects of Chemical and Mechanical Sagebrush Control. Progress Report for Period Ending 6/30/70. W-105-R-3, 4, 5. Montana Department of Fish and Game, Game Management Research Bureau, Bozeman, Montana.

Katz, M., and A.W. McCallum. 1939. Effects of Sulfur Dioxide on Vegetation. NRC No. 815. National Research Council of Canada, Ottawa, Ontario. 447 pp.

Khan, A.G. 1972. The Effect of VA Mycorrhizal Associations on Growth of Cereals. I. Effects on Maize Growth. New Phytol., 71:613-619.

Kinden, D.A., and M.F. Brown. 1975. Electron Microscopy of Vesicular-Arbuscular Mycorrhizae of Yellow Poplar. II. Intracellular Hyphae and Vesicles. Can J. Microbiol., 21:1768-1780.

Kleinschmidt, G.D., and J.W. Gerdemann. 1972. Stunting of Citrus Seedlings in Fumigated Nursery Soils Related to the Absence of Endomycorrhiza. Phytopath., 62:1447-1453.

Linzon, S.N. 1973. The Effects of Air Pollution on Forests. Presented at the Fourth Joint Chemical Engineering Conference. 18 pp.

Maguire, J.D. 1962. Speed of Germination-Aid in Selection and Evaluation for Seedling Emergence and Vigor. Crop Science, 2:176-177.

Martin, H. 1959. The Scientific Principles of Crop Protection. Arnold Press, London. 359 pp.

Marx, D.H. 1972. Ectomycorrhizae as Biological Deterrent to Pathogenic Root Infections. Ann. Rev. Phytopath., 10:429-454.

McGovern, P.C., and D. Balsillie. 1974. Effects of Sulphur Dioxide and Heavy Metals on Vegetation in the Sudbury Area (1973). Ontario Ministry of the Environment, Sudbury, Ontario. 47 pp.

Mejstrik, V.K. 1972. Vesicular-Arbuscular Mycorrhizas of the Species of a *Molinietum coeruleae* L.I. Association: The Ecology. New Phytol., 74:883-890.

Miles, P.W. 1968. Insect Secretions in Plants. Ann. Rev. Phytopath., 6:137-164.

Miller, C.O. 1967. Zeatin and Zeatin Riboside from a Mycorrhizal Fungus. Science, 157:1055-1057.

Montana Department of Health and Environmental Sciences. 1978. Annual Air Quality Data Summary for Montana for 1977, J. Gelhaus, ed. Air Quality Bureau, Environmental Sciences Division, Helena, Montana. 89 pp.

Morley, C.D., and B. Mosse. 1976. Abnormal Vesicular-Arbuscular Mycorrhizal Infections in White Clover Induced by Lupin. Trans. Brit. Mycol. Soc., 67(3):510-513.

Morrison, T.M. 1962. Uptake of Sulphur by Mycorrhizal Plants. New Phytol., 61:21-27.

Mosse, B. 1973. Advances in the Study of Vesicular-Arbuscular Mycorrhiza. Ann. Rev. Phytopath., 11:171-196.

________. 1977. Plant Growth Responses to Vesicular-Arbuscular Mycorrhiza. X. Responses of Stylosanthes and Maize to Inoculation in Unsterile Soils. New Phytol., 78:277-288.

Murdoch, C.L., J.A. Jacobs, and J.W. Gerdemann. 1967. Utilization of Phosphorous Sources of Different Availability by Mycorrhizal and Non-mycorrhizal Maize. Plant Soil, 27:329-334.

Nicolson, T.H. 1959. Mycorrhiza in the Gramineae. I. Vesicular-Arbuscular Endophytes, with Special Reference to the External Phase. Trans. Brit. Mycol. Soc., 42:421-438.

________. 1960. Mycorrhiza in the Granineae. II. Development in Different Habitats, Particularly Sand Dunes. Trans. Brit. Mycol. Soc., 43:132-145.

Northern Cheyenne Tribe. 1976. The Northern Cheyenne Air Quality Redesignation Report and Request. Northern Cheyenne Tribe, Inc., Lame Deer, Montana. 190 pp.

Osberg, T.R. 1978. Personal Communication. U.S. Environmental Protection Agency, Environmental Photographic Interpretation Center, Warrenton, Virginia.

Read, D.J., H.K. Koucheki, and J. Hodgson. 1976. Vesicular-Arbuscular Mycorrhiza in Natural Vegetation Systems. I. The Occurrence of Infection. New Phytol., 77:641-653.

Rich, J.R., and G.W. Bird. 1974. Association of Early-Season Vesicular-Arbuscular Mycorrhizae with Increased Growth and Development of Cotton. Phytopath., 64(11):1421-1425.

Richards, B.N., and G.K. Voigt. 1964. Role of Mycorrhiza in Nitrogen Fixation. Nature, 201:310-311.

Ross, J.P. 1971. Effect of Phosphate Fertilization in Yield of Mycorrhizal and Non-mycorrhizal Soybean. Phytopath., 61:1400-1403.

Russell, E.W. 1973. Soil Conditions and Plant Growth. Tenth Edition. Longman, New York. 849 pp.

Saif, S.R. 1977. The Influence of Stage of Host Development on Vesicular-Arbuscular Mycorrhizae and Endogonaceous Spore Population in Field-grown Vegetable Crops. I. Summer-grown Crops. New Phytol., 79:341-348.

Sanders, F.E., and P.B. Tinker. 1973. Phosphate Flows into Mycorrhizal Roots. Pest. Sci., 4:385.

________, R.L.B. Black, and S.M. Palmerley. 1977. The Development of Endomycorrhizal Root Systems: I. Spread of Infection and Growth Promoting Effects with Four Species of Vesicular-Arbuscular Endophyte. New Phytol., 78:257-268.

Sanni, S.O. 1976a. Vesicular-Arbuscular Mycorrhizae in Some Nigerian Soils. The Effect of Gigaspora Gigantea on the Growth of Rice. New Phytol., 77:673-674.

________. 1976b. Vesicular-Arbuscular Mycorrhiza in Some Nigerian Soils and Their Effect on the Growth of Cowpea (*Vigna unguiculata*), Tomato (*Lycopersicon esculentum*) and Maize (*Zea mays*). New Phytol., 77:667-671.

Schott, J.R., D.W. Gaucher, and J.E. Walker. 1976. Aerial Photographic Technique for Measuring Vegetation Stress from Sulfur Dioxide. Report No. YB-5967-M-1. Calspan Corporation, Buffalo, New York.

Shterenberg, P.M., and P.N. Kostyak. 1967. Seventy Years Since the Discovery of Plant Mycotrophy by F.M. Kamenskii. In: Mycotrophy in Plants, A.A. Imshenetskii, ed. Academy of Sciences of the USSR, Institute of Microbiology. 362 pp.

Slankis, V. 1974. Soil Factors Influencing Formation of Mycorrhizae. Ann. Rev. Phytopath., 12:437-458.

Smirnov, N.V. 1939. Estimates of Deviation Between Empirical Distribution Functions in Two Independent Samples. Bull. Moscow University, 2(2):3-16.

Snedecor, G.W. 1956. Statistical Methods. Fifth Edition. Iowa State College Press, Ames, Iowa. 534 pp.

Sobotka, A. 1964. Vliv Prumyslovych Exhalatuna Pudni. Zivenu Smrkovjch Porostu Krusnych hor. Lesn. Cas. Praha, 10:987-1002.

Sokal, R.R., and F.J. Rohlf. 1969. Biometry. The Principles and Practice of Statistics in Biological Research. W.H. Freeman & Company, San Francisco, California. 776 pp.

Sparling, G.P., and P.B. Tinker. 1975. Mycorrhizas in Penine Grassland. In: Endomycorrhizas, F.E. Sanders, B. Mosse, and P.B. Tinker, eds. Academic Press, London. pp. 545-560.

Taylor, J.E. 1976. Personal Communication. Department of Animal and Range Sciences, Montana State University, Bozeman, Montana.

Tingey, D.T., L. Bard, and R.W. Field. 1978. The Relative Sensitivity of Selected Plant Species to Several Pollutants Singly and in Combination. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Third Interim Report, Colstrip, Montana, December, 1977, E.M. Preston and R.A. Lewis, eds. EPA-600/3-78-021, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 508-513.

Treshow, M. 1968. The Impact of Air Pollutants on Plant Populations. Phytopath., 58:1108-1113.

________. 1975. Interaction of Air Pollutants and Plant Disease. In: Responses of Plants to Air Pollution, J.B. Mudd and T.T. Kozlowski, eds. Academic Press, New York. pp. 307-334.

Trinick, M.J. 1977. Vesicular-Arbuscular Infection and Soil Phosphorous Utilization in *Lupinus* spp. New Phytol., 78:297-304.

Van Bruggen, T. 1976. The Vascular Plants of South Dakota. The Iowa State University Press, Ames, Iowa. 538 pp.

Voigt, G.K. 1969. Mycorrhizae and Nutrient Mobilization. In: Mycorrhizae--Proceedings of the First North American Conference on Mycorrhizae. Miscellaneous Publication 1189. USDA Forest Service. pp. 122-131.

Wheeler, H., and P. Hanchey. 1968. Permeability Phenomena in Plant Disease. Rev. Phytopath., 6:331-350.

Zak, B. 1964. Role of Mycorrhizae in Root Disease. Ann. Rev. Phytopath., 2:377-392.

Ziegler, I. 1975. The Effect of SO_2 Pollution on Plant Metabolism. Residue Reviews, 56:79-105.

APPENDIX TABLE 14.1. EFFECTS OF REMOVING OUTLYING SULFUR OBSERVATIONS ON MEAN VALUES AND 95% CONFIDENCE INTERVALS[*] FOR TOTAL SULFUR AT THE OFF PLOT CONTROL (OPC) AND ZAPS IA ON SEPTEMBER 11, 1977.

OPC		IA	
Sample	ppm S	Sample	ppm S
1	750	8NW	1,050
2	800	8SE	950
3	900	9NE	1,350
4	950	10NE[†]	1,950
5	800	12SE	900
6	850	14NW	950
7	900	17SE	1,000
8	850	19NE	800
9	800	12NW	1,150
10[†]	2,150	20SE	1,000

Mean Values[‡] and 95% Confidence Intervals

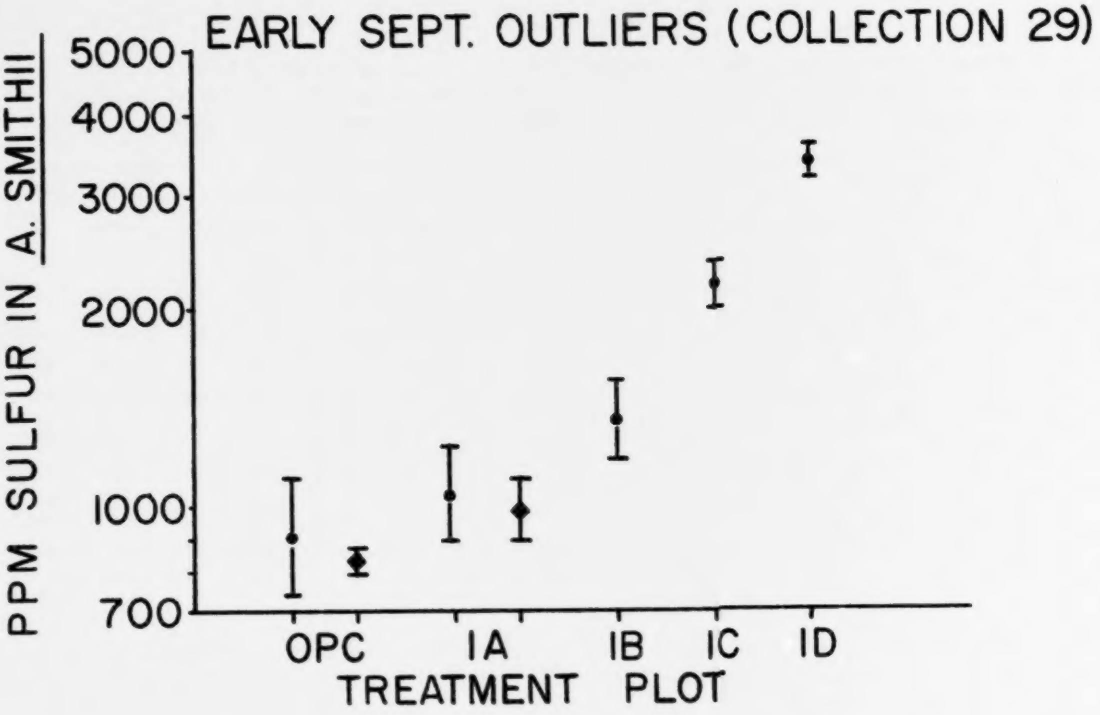
OPC including sample 10	IA including sample 10NE
$\bar{x} =$ 922	$\bar{x} =$ 1,069
$\bar{x} + t_{s\bar{x}} =$ 1,141	$\bar{x} + t_{s\bar{x}} =$ 1,268
$\bar{x} - t_{s\bar{x}} =$ 746	$\bar{x} - t_{s\bar{x}} =$ 901
OPC excluding sample 10	**IA excluding sample 10NE**
$\bar{x} =$ 842	$\bar{x} =$ 1,006
$\bar{x} + t_{s\bar{x}} =$ 892	$\bar{x} + t_{s\bar{x}} =$ 1,128
$\bar{x} - t_{s\bar{x}} =$ 795	$\bar{x} - t_{s\bar{x}} =$ 897

---

[*] $\bar{x} \pm t_{.05} s_{\bar{x}}$

[†] These values were statistical outliers according to Dixon's Criteria at the 95% significance level after: Grubbs, Frank E. 1969. Procedures for Detecting Outlying Observations in Samples. Technometrics, 2(1).

[‡] Statistics shown are based on $\log_{10}$ transformations of the raw data.



Appendix Figure 14.1. Means and 95% confidence limits ($\log_{10}$) of total sulfur in *A. smithii*; outliers removed are indicated by ◆ .

APPENDIX TABLE 14.2a. SULFUR (PPM) IN LIVE AND DEAD PLANT MATERIAL COLLECTED FROM ZAPS I, TREATMENT A, IN 1976.

Collection Date	Julian Date	*A. smithii*		*K. cristata*		*P. sandbergii*		*A. longiseta*		*A. millefolium*		*A. frigida*	
		Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead
April 6	97	1,300	500	1,675	600	ns	ns	ns	400	800	700	1,100	1,100
May 13	134	700	ns	700	ns	500	ns	ns	ns	600	ns	1,250	ns
June 1	153	850	ns	550	ns	550	ns	ns	ns	550	ns	--	ns
June 18	170	1,000	ns	900	ns	550	ns	ns	ns	850	ns	1,550	ns
July 4	186	700	500	650	550	ns	400	1,000	ns	550	550	1,300	ns
August 2	215	1,000	900	800	500	ns	ns	750	700	550	650	1,250	ns
August 16	229	850	750	700	550	ns	ns	950	850	750	600	1,300	1,300
Sept 1	245	1,275	1,225	875	750	ns	ns	900	600	900	800	1,550	1,300
Sept 17	261	745 (731)	800 (790)	575 (568)	590 (581)	ns	ns	800	650	650	650	1,300	950
Oct 16	290	850	750	800	800	ns	ns	ns	650	650	600	1,350	1,150

ns = not sampled

-- = missing sample

( ) = antilog log $\overline{x}$

APPENDIX TABLE 14.2b. SULFUR (PPM) IN LIVE AND DEAD PLANT MATERIAL COLLECTED FROM ZAPS II, TREATMENT A, IN 1976.

Collection Date	Julian Date	*A. smithii*		*K. cristata*		*P. sandbergii*		*S. viridula*		*A. millefolium*		*A. frigida*	
		Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead
April 10	101	1,716	350	1,575	500	ns	ns	ns	550	1,175	550	1,600	750
May 8	128	800	ns	650	ns	650	ns	800	ns	850	ns	1,550	ns
May 31	152	850	ns	750	ns	600	ns	800	ns	700	ns	913	ns
June 18	170	1,050	ns	900	ns	700	ns	1,100	ns	950	ns	1,350	ns
July 6	188	1,050	ns	500	ns	ns	450	950	ns	650	650	1,133	ns
August 3	216	800	800	650	550	ns	ns	1,150	950	600	700	1,250	ns
August 15	228	950	850	650	700	ns	ns	1,000	750	850	700	1,350	1,050
Sept 1	245	800	850	850	650	ns	ns	1,100	850	750	600	1,550	750
Sept 19	263	745 (735)	830 (814)	800 (790)	519 (502)	ns	ns	1,050	800	900	1,000	1,550	1,200

ns = not sampled

( ) = antilog log $\overline{x}$

APPENDIX TABLE 14.2c. SULFUR (PPM) IN LIVE AND DEAD PLANT MATERIAL COLLECTED FROM ZAPS I, TREATMENT B, IN 1976.

Collection Date	Julian Date	*A. smithii*		*K. cristata*		*P. sandbergii*		*A. longiseta*		*A. millefolium*		*A. frigida*	
		Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead
April 7	98	1,400	600	1,575	500	ns	ns	ns	725	1,275	—	1,700	1,150
May 13	134	1,050	ns	900	ns	950	ns	ns	ns	800	ns	1,950	ns
June 1	153	1,050	ns	900	ns	783	ns	ns	ns	800	ns	1,450	ns
June 16	168	1,350	ns	1,425	ns	850	ns	ns	ns	1,200	ns	1,850	ns
July 5	187	1,450	850	1,200	1,050	ns	500	850	ns	700	1,050	1,750	ns
August 2	215	1,500	1,400	1,850	1,400	ns	ns	1,050	1,050	1,200	1,350	1,933	ns
August 16	229	1,700	1,650	1,550	1,550	ns	ns	1,200	1,050	1,150	1,400	1,600	1,600
Sept 2	246	1,650	2,000	1,800	1,600	ns	ns	1,300	1,150	950	1,225	1,500	1,900
Sept 17	261	1,565 (1,538)	1,605 (1,549)	1,585 (1,525)	1,311 (1,286)	ns	ns	1,000	1,000	1,000	1,250	1,500	1,600
Oct 16	290	1,200	1,450	1,250	1,250	ns	ns	ns	950	1,300	1,300	1,950	1,650

ns = not sampled — = missing sample ( ) = antilog log $\overline{x}$

APPENDIX TABLE 14.2d. SULFUR (PPM) IN LIVE AND DEAD PLANT MATERIAL COLLECTED FROM ZAPS II, TREATMENT B, IN 1976.

Collection Date	Julian Date	*A. smithii*		*K. cristata*		*P. sandbergii*		*S. viridula*		*A. millefolium*		*A. frigida*	
		Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead
April 10	101	1,250	600	1,050	550	ns	ns	ns	—	850	700	2,125	1,050
May 11	132	1,110	ns	800	ns	975	ns	1,275	ns	1,050	ns	1,350	ns
June 1	153	1,300	ns	1,100	ns	800	ns	950	ns	1,050	ns	1,050	ns
June 21	173	1,450	ns	850	ns	950	ns	1,350	ns	1,800	ns	1,900	ns
July 7	189	1,600	ns	1,300	ns	ns	850	1,600	ns	1,100	1,400	1,950	ns
August 3	216	1,550	1,900	1,550	1,350	ns	ns	1,300	1,200	1,300	1,550	1,750	ns
August 15	228	1,250	1,500	1,050	1,250	ns	ns	1,100	1,350	775	1,300	1,550	1,350
Sept 2	246	1,450	1,950	1,350	1,300	ns	ns	1,600	1,500	1,350	1,350	1,800	1,500
Sept 18	262	1,170 (1,122)	1,445 (1,429)	1,069 (1,044)	912 (903)	ns	ns	1,300	1,600	1,100	1,150	2,150	750

ns = not sampled — = missing sample ( ) = antilog log $\overline{x}$

APPENDIX TABLE 14.2e. SULFUR (PPM) IN LIVE AND DEAD PLANT MATERIAL COLLECTED FROM ZAPS I, TREATMENT C, IN 1976.

Collection Date	Julian Date	*A. smithii*		*K. cristata*		*P. sandbergii*		*A. longiseta*		*A. millefolium*		*A. frigida*	
		Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead
April 7	98	1,300	800	1,200	850	ns	ns	ns	750	900	1,050	2,700	1,100
May 13	134	1,350	ns	1,200	ns	1,150	ns	ns	ns	1,150	ns	2,050	ns
June 1	153	1,500	ns	1,600	ns	1,450	ns	ns	ns	1,450	ns	2,050	ns
June 16	168	2,050	ns	2,250	ns	988	ns	ns	ns	1,650	ns	—	ns
July 5	187	1,750	1,200	2,100	1,250	ns	900	1,450	ns	2,000	2,700	3,100	ns
August 2	215	2,550	2,750	2,350	2,200	ns	ns	1,250	1,400	2,200	2,850	2,700	ns
August 16	229	2,550	2,950	2,400	2,000	ns	ns	1,750	1,700	1,850	2,150	2,350	2,325
Sept 2	246	2,225	2,800	2,600	1,800	ns	ns	1,275	1,500	2,150	2,350	3,150	2,100
Sept 17	261	2,235 (2,143)	1,990 (1,961)	1,585 (1,554)	1,260 (1,244)	ns	ns	1,550	1,250	2,000	1,950	3,200	1,350
Oct 16	290	1,950	1,800	2,050	1,550	ns	ns	ns	1,150	2,500	1,950	3,100	2,800

ns = not sampled — = missing sample ( ) = antilog log $\bar{x}$

APPENDIX TABLE 14.2f. SULFUR (PPM) IN LIVE AND DEAD PLANT MATERIAL COLLECTED FROM ZAPS II, TREATMENT C, IN 1976.

Collection Date	Julian Date	*A. smithii*		*K. cristata*		*P. sandbergii*		*S. viridula*		*A. millefolium*		*A. frigida*	
		Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead
April 9	100	1,100	400	1,675	550	ns	ns	ns	450	550	500	1,250	800
May 11	132	1,150	ns	1,550	ns	1,450	ns	2,500	ns	1,400	ns	1,650	ns
May 31	152	1,700	ns	1,300	ns	1,250	ns	1,600	ns	1,200	ns	1,750	ns
June 21	173	2,200	ns	2,250	ns	1,250	ns	2,250	ns	2,550	ns	—	ns
July 7	189	2,150	ns	2,450	ns	ns	1,100	2,600	ns	2,200	2,450	2,325	ns
August 3	216	2,616	2,850	2,650	2,250	ns	ns	2,100	2,500	2,350	3,750	3,150	ns
August 15	228	2,200	3,400	2,250	1,950	ns	ns	2,250	2,250	2,150	2,800	3,050	3,350
Sept 2	246	2,450	3,200	2,550	2,050	ns	ns	2,950	2,800	1,800	2,250	3,950	2,800
Sept 18	262	1,925 (1,820)	2,630 (2,505	1,775 (1,731)	2,094 (1,956)	ns	ns	2,000	2,450	2,725	2,100	2,550	2,725

ns = not sampled — = missing sample ( ) = antilog log $\bar{x}$

APPENDIX TABLE 14.2g. SULFUR (PPM) IN LIVE AND DEAD PLANT MATERIAL COLLECTED FROM ZAPS I, TREATMENT D, IN 1976.

Collection Date	Julian Date	*A. smithii*		*K. cristata*		*P. sandbergii*		*A. longiseta*		*A. millefolium*		*A. frigida*	
		Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead
April 8	99	1,500	1,000	1,200	1,050	ns	ns	ns	800	1,350	1,600	2,650	2,725
May 13	134	1,750	ns	1,650	ns	1,550	ns	ns	ns	1,300	ns	3,300	ns
June 1	153	1,950	ns	2,017	ns	1,500	ns	ns	ns	2,750	ns	3,400	ns
June 16	168	2,400	ns	3,350	ns	1,900	ns	ns	ns	3,600	ns	—	ns
July 4	186	2,600	1,519	3,850	2,100	ns	2,350	1,850	ns	3,725	5,750	5,150	ns
August 2	215	3,175	4,725	4,275	2,850	ns	ns	2,100	1,700	3,900	5,250	5,450	ns
August 16	229	3,450	3,200	3,400	2,350	ns	ns	2,350	1,800	4,400	6,367	4,250	4,200
Sept 2	246	3,800	4,100	3,400	2,650	ns	ns	2,400	2,100	5,325	4,750	5,550	3,850
Sept 17	261	3,105 (3,059)	2,700 (2,639)	3,210 (3,150)	2,145 (2,115)	ns	ns	2,050	1,950	4,450	4,350	5,800	3,400
Oct 16	290	3,750	2,700	2,700	2,100	ns	ns	ns	1,900	4,750	2,700	5,250	4,300

ns = not sampled — = missing sample ( ) = antilog log $\bar{x}$

APPENDIX TABLE 14.2h. SULFUR (PPM) IN LIVE AND DEAD PLANT MATERIAL COLLECTED FROM ZAPS II, TREATMENT D, IN 1976.

Collection Date	Julian Date	*A. smithii*		*K. cristata*		*P. sandbergii*		*S. viridula*		*A. millefolium*		*A. frigida*	
		Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead
April 9	100	1,400	450	1,625	600	ns	ns	ns	500	500	550	1,450	850
May 13	134	1,850	ns	1,300	ns	1,400	ns	3,250	ns	2,600	ns	2,700	ns
May 31	152	3,250	ns	1,550	ns	1,900	ns	2,150	ns	2,800	ns	3,550	ns
June 21	173	4,350	ns	3,750	ns	2,400	ns	4,650	ns	4,983	ns	—	ns
July 7	189	4,200	ns	4,183	ns	ns	1,800	4,850	ns	3,875	4,750	4,850	ns
August 3	216	4,150	5,675	3,550	3,250	ns	ns	3,600	3,000	4,100	5,400	5,150	ns
August 15	228	3,100	3,950	3,550	3,800	ns	ns	4,150	3,800	4,950	5,500	5,450	5,925
Sept 2	246	3,950	4,450	3,950	2,900	ns	ns	4,250	4,000	6,000	4,550	5,100	4,350
Sept 18	262	2,618 (3,150)	4,135 (4,068)	3,223 (3,101)	2,511 (2,467)	ns	ns	3,425	3,250	4,150	2,850	4,800	5,650

ns = not sampled — = missing sample ( ) = antilog log $\bar{x}$

APPENDIX TABLE 14.3. SULFUR LEVELS (PPM) IN *AGROPYRON SMITHII* DURING 1977.

Collection No. and Date		OPC	IA	IIA	IB	IIB	IC	IIC	ID	IID
21, late April	Julian Day	114	112	112	113	112	113	111	113	111
	$\bar{x}$	1,198	1,448	1,466	1,620	1,580	1,590	1,596	1,893	2,073
	n	10	9	10	10	8	8	10	10	9
	l_1	1,082	1,248	1,295	1,419	1,406	1,404	1,424	1,689	1,854
	l_2	1,328	1,680	1,659	1,850	1,776	1,802	1,789	2,123	2,319
22, mid-May	Julian Day	134	132	130	133	131	133	131	133	132
	$\bar{x}$	871	1,081	1,538	1,285	1,810	2,081	1,912	1,937	2,351
	n	10	9	9	9	10	10	10	6	9
	l_1	786	981	1,435	1,169	1,712	1,986	1,758	1,700	2,053
	l_2	965	1,191	1,649	1,413	1,904	2,179	2,080	2,207	2,693
23, late May	Julian Day	149	148	148	148	147	148	147	148	147
	$\bar{x}$	1,132	1,172	1,212	1,421	1,517	1,617	1,917	2,142	2,815
	n	10	10	10	10	10	10	10	10	10
	l_1	974	1,116	1,112	1,345	1,425	1,447	1,829	1,945	2,550
	l_2	1,314	1,231	1,319	1,501	1,614	1,807	2,121	2,360	3,107
24, mid-June	Julian Day	168	166	166	167	166	167	165	167	165
	$\bar{x}$	1,197	1,050	1,209	1,359	1,548	2,031	2,139	2,590	3,198
	n	10	10	10	10	10	10	10	10	10
	l_1	1,057	981	1,126	1,215	1,443	1,819	2,027	2,308	2,903
	l_2	1,355	1,124	1,299	1,521	1,660	2,267	2,257	2,906	3,523
25, early July	Julian Day	185	183	182	184	182	184	182	184	183
	$\bar{x}$	1,073	1,011	1,184	1,282	1,536	1,972	2,403	2,993	3,916
	n	7	10	10	9	10	10	10	10	10
	l_1	905	796	1,098	1,122	1,366	1,716	2,059	2,617	3,548
	l_2	1,273	1,285	1,275	1,465	1,726	2,266	2,805	3,424	4,322
26, mid-July	Julian Day	201	200	199	200	199	201	199	201	200
	$\bar{x}$	1,129	1,202	1,037	1,443	1,440	2,013	2,166	2,832	3,376
	n	10	10	10	10	10	10	10	10	10
	l_1	965	1,068	979	1,345	1,294	1,778	1,980	2,590	3,050
	l_2	1,321	1,352	1,097	1,549	1,602	2,278	2,371	3,098	3,738
27, early August	Julian Day	216	217	215	217	216	218	216	218	215
	$\bar{x}$	883	880	1,224	1,288	1,434	2,251	2,304	3,315	3,466
	n	8	10	10	10	9	10	10	10	10
	l_1	763	817	1,142	1,161	1,303	1,998	2,035	2,913	3,090
	l_2	1,022	949	1,312	1,428	1,579	2,536	2,607	3,772	3,887
28, late August	Julian Day	236	234	233	235	233	235	233	236	234
	$\bar{x}$	1,197	1,200	1,038	1,510	1,508	2,441	2,396	3,559	3,572
	n	10	10	10	10	10	10	10	10	10
	l_1	1,071	1,019	892	1,360	1,379	2,083	2,140	3,113	3,205
	l_2	1,338	1,414	1,208	1,677	1,650	2,860	2,681	4,068	3,982
29, early Sept	Julian Day	254	253	251	253	252	253	252	254	252
	$\bar{x}$	842	1,006	905	1,382	1,293	2,234	2,222	3,480	3,253
	n	9	9	10	10	10	10	10	10	10
	l_1	795	897	810	1,193	1,169	2,045	1,921	3,253	2,680
	l_2	892	1,128	1,010	1,602	1,431	2,441	2,571	3,724	3,948
30, late Sept	Julian Day	278	272	269	276	270	276	270	276	271
	$\bar{x}$	1,066	1,276	840	1,332	1,362	2,149	2,243	3,322	3,288
	n	10	10	10	10	10	10	10	10	10
	l_1	948	1,060	732	1,124	1,235	1,842	2,021	2,989	2,813
	l_2	1,199	1,536	965	1,578	1,503	2,508	2,489	3,692	3,842
31, late Oct	Julian Day	299	300	290	300	291	299	291	299	292
	$\bar{x}$	1,917	1,643	1,675	1,325	1,469	2,275	1,930	2,275	3,132
	n	3	5	2	2	10	2	7	2	9
	l_1		1,279			1,308		1,722		268
	l_2		2,111			1,650		2,163		3,648

NOTE: Limits are the 95% level; $\log_{10}$ transformation applied when $n > 5$.

APPENDIX TABLE 14.4a. PERCENT MYCORRHIZAL INFECTION (% M) AND MEAN ABSORBANCE (ABS) FOR COLLECTION 26 DURING MID-JULY, 1977.

Plots ↓	← Subplots →									
	1 7NW	2 8NE	3 9SW	4 9NW	5 10NW	6 12SW	7 14NE	8 17NW	9 18SE	10 19SW
OPC										
% M	62.16	56.25	62.50		46.88	75.00	56.76	79.17	80.00	78.57
ABS	.380	.305	.290	.450	.300	.330	.315	.335	.550	.320
IA										
% M	50.00		70.83	63.64	62.50	53.85	58.62	51.62	53.06	57.14
ABS	.205	.275	.205	.295	.205	.260	.190	.200	.300	.190
IB										
% M	42.25	72.41	69.56	70.83	50.82	68.18	78.57	65.22	68.75	62.50
ABS	.245	.190	.210	.190	.235	.190	.225	.260	.225	.225
IC										
% M	78.57	57.14	50.00	54.29	59.46	52.17	52.63	50.00	61.11	43.33
ABS	.280	.255	.255	.260	.260	.235	.300	.230	.305	.305
ID										
% M	40.62	60.00	53.66	62.96	65.71	72.41	58.62	72.84	75.00	59.46
ABS	.230	.230	.275	.350	.230	.265	.230	.275	.225	.225
IIA										
% M	52.94	57.78	46.15	56.41	56.25	45.45	68.18	58.82	66.67	67.65
ABS	.435	.680	.360	.440	.385	.370	.385	.325	.370	
IIB										
% M	48.57	57.90	57.14	78.57	64.86	33.33	47.83	69.44	67.50	51.85
ABS	.290	.450	.355	.380	.390	.365		.305	.380	.400
IIC										
% M	29.41	29.41	26.53	54.54	43.75	28.89	38.78	51.26	23.07	23.91
ABS	.435	.440	.185		.390	.405	.365		.395	.375
IID										
% M	34.62	60.87	63.33	56.52	35.90	61.54	54.17	27.50	51.61	37.50
ABS	.400	.375	.310	.250	.325	.350	.435	.315	.315	.270

APPENDIX TABLE 14.4b. PERCENT MYCORRHIZAL INFECTION (% M) AND MEAN ABSORBANCE (ABS) FOR COLLECTION 29 DURING MID-SEPTEMBER, 1977.

Plots ↓	← Subplots →									
	1 5E	2 8NW	3 9NE	4 10NE	5 12SE	6 14NW	7 17SE	8 19NW	9 19NE	10 20SE
OPC										
% M	57.70	61.11	47.06	45.83	50.00	26.67	25.00	30.00	41.02	20.83
ABS	.425	.330	.230	.275	.320	.235	.200	.230	.318	.220
IIA										
% M	76.66		68.00	56.76	69.51		69.56	48.28	40.86	66.66
ABS	.285		.320	.240	.290		.268	.272	.268	.300
IIB										
% M	52.42	63.93	44.83	55.55	51.67	43.64	56.60		52.63	56.25
ABS	.275	.290	.290	.235	.355	.265	.335	.285	.330	.315
IIC										
% M	42.37	41.97			47.37	42.59	39.47	42.22	39.39	
ABS	.308	.235		.235	.330	.290	.295	.285	.225	
IID										
% M	19.23	13.95	19.12	40.00	19.56	25.46		17.65		26.19
ABS	.245	.200	.248	.235	.245	.225		.205	.260	.210

SECTION 15

RESPONSE OF SELECTED SMALL GRAINS, RANGE GRASSES AND ALFALFA TO SULFUR DIOXIDE

R. G. Wilhour, G. E. Neely, D. E. Weber and L. C. Grothaus

ABSTRACT

Experiments were conducted to determine the effects of various sulfur dioxide (SO_2) treatments on yield of small grains (spring wheat, *Triticum aestivum* 'Olaf'; Durum wheat, *Triticum tirgidum* 'Ward'; barley, *Hordeum vulgare* 'Hector') and alfalfa (*Medicago sativa* 'Ladak-65') growing in a field environment. The experiments were: 1) chronic exposure experiment to determine the effects of weekly exposure to SO_2 treatments of 0, 3, 5, 10 or 15 pphm for 72 continuous hr; 2) multiple exposure experiment to determine the effects of varying the frequency of exposure to SO_2 treatments of 0, 25, 40, 80 or 120 pphm for 3 hr; and 3) a growth stage experiment to determine if phenological stages exist during which plants are most sensitive to a single SO_2 treatment of 0, 25, 40, 80 or 120 pphm for 3 hr. Another study was included to determine the effects of 3 hr treatments to 0, 25, 40, 80 or 120 pphm SO_2 repeated at two-week intervals on the yields of range grasses (crested wheatgrass, *Agropyron desertorum* 'Nordan'; western wheatgrass, *Agropyron smithii*; Russian wild ryegrass, *Elymus junceus*; blue grama grass, *Bouteloua gracilis*; needle and thread grass, *Stipa comata*) and alfalfa grown in containers.

The yields of Durum wheat and barley treated weekly with 15 pphm SO_2 for 72 continuous hr were 42 and 44% of the controls, respectively. These results were not significant at the 95% probability level, but the data suggested that weekly, 72-hr exposures to SO_2 concentrations as low as 10-15 pphm could suppress yields. The yield of spring wheat was not affected by a similar treatment. Varying the frequency of 3-hr exposures to SO_2 concentrations up to 120 pphm from as often as once every week to as infrequently as once every 5 weeks had no effect on yields of the small grains and alfalfa. No growth stage was identified during which the small grains or alfalfa were most sensitive to a single 3-hr exposure to SO_2 concentrations up to 120 pphm. The growth of tops and roots of range grasses and alfalfa were not affected by 3-hr exposures to concentrations of SO_2 up to 120 pphm repeated at 14-day intervals during September and October.

INTRODUCTION

The increased reliance of the United States on energy produced by coal-fired power plants will result in elevated concentrations of SO_2 in areas sharing airsheds with electrical generating stations. The Poplar River Valley of Montana, due to the current and planned construction of coal-fired power plants in neighboring states and Canada, will experience increased SO_2 concentrations. This study was initiated due to the concern of EPA Region VIII in Denver, Colorado regarding the potential detrimental effects of elevated SO_2 concentrations on agricultural crops important to the economy of the Poplar River Valley.

Several studies have described injury symptoms (Lamb, 1972; Hill *et al.*, 1974; Tingey *et al.*, 1978), changes in various plant processes (Bennett and Hill, 1973; Rabe and Kreeb, 1976; Neely *et al.*, 1977; Dodd, Section 13 of this report) and yield effects (Davis *et al.*, 1966; Booth *et al.*, 1976; Brough and Parry, 1976; Heitschmidt, 1977; Neely *et al.*, 1977; Dodd, Section 13 of this report) of SO_2 on plants similar to those economically important in the Poplar River Valley. Although these studies demonstrated probable response characteristics of several plant species to SO_2 and provided some dose/response data, knowledge remained incomplete for a reasonable assessment of the effects of increased SO_2 levels on the agricultural economy of the Poplar River Valley.

A research program was planned to broaden the knowledge concerning the response of important plant species grown in the Poplar River Valley to SO_2. Two studies were conducted utilizing different species and emphasizing different objectives. Small grains and alfalfa were included in a study which had three objectives: 1) to determine if more frequent exposure to given concentrations of SO_2 resulted in greater yield reductions; 2) to assess the effects of a range of SO_2 concentrations on yield; and 3) to determine if periods of time exist during which a species is more sensitive to SO_2. Another study utilized native range grasses and alfalfa and was designed to assess the effects of a range of SO_2 concentrations on yield.

MATERIALS AND METHODS

The experimental SO_2 exposures were conducted at the Schmidt research farm which is located approximately 5 miles east of Corvallis, Oregon. The farm is owned and operated by Oregon State University.

Field Exposure Study

Three distinctive experiments were included in this study. A chronic exposure experiment was conducted to determine the effects on yield of small grains (spring wheat, *Triticum aestivum* 'Olaf'; Durum wheat, *Triticum turgidum* 'Ward'; and barley, *Hordeum vulgare* 'Hector') and alfalfa (*Medicago sativa* 'Ladak-65') from repeated, lengthy exposures to low concentrations of SO_2. Treatments in this experiment were ambient air (never covered by an exposure chamber), 0 (ambient air filtered by activated charcoal), 3, 5, 10, or 15 pphm SO_2 (ambient air filtered by activated charcoal amended with desired SO_2 concentrations). The duration of the SO_2 treatments was 72 hr (Friday noon until Monday noon) and the treatments were repeated each week for 12 weeks beginning May 6, 1977.

The objectives of the multiple exposure experiment were to correlate yields of small grains and alfalfa with 1) SO_2 concentrations in the range of 0-120 pphm and 2) frequency of exposure to these SO_2 concentrations. The effects of all combinations of five SO_2 concentrations (0, 25, 40, 80 or 120 pphm) and seven exposure frequencies (ranging from once per week to once every five weeks*) were examined as they affect plant yields. The SO_2 treatments lasted 3 hr beginning at 8 a.m. The experiment was continued for 12 weeks, beginning May 11.

A growth stage experiment was conducted to determine if phenological stages of development exist during which a single exposure to SO_2 concentrations of 0, 25, 40, 80 or 120 pphm most affects yield of small grains or alfalfa. Treatments representing all combinations of five SO_2 concentrations (0, 25, 40, 80 or 120 pphm) and six growth stages (two-week intervals) were used in this experiment. The SO_2 treatments were 3 hr in duration (8-11 a.m.) and an experimental plot was treated only once during the experiment. A different group of five experimental plots (one for each SO_2 concentration) was treated at 2-week intervals, beginning May 17.

The soil at the Schmidt research farm is a Willamette silt loam. The surface layer is a well drained, very dark brown silt loam, about 60 cm thick over a silty clay loam subsoil. A chemical analysis of the soil prior to seeding identified the following chemical concentrations: nitrate (6.6 ppm), phosphorus (45 ppm), potassium (252 ppm), sulfur (6.2 ppm) and boron (.42 ppm). The soil had a pH of 5.8, cation exchange capacity of 18.35 milliequivalents per 100 grams and an organic matter content of 3.29%. The following amendments were applied and incorporated into the soil prior to seeding: 16-20-0-14 (N-P-K-S) and 0-0-52-17 at rates of 337 and 129 kg/ha, respectively, boron (14.3%) at 3 kg/ha and agriculture limestone at 8980 kg/ha. The amendments and rates were selected to provide an average growing condition capable of adequately sustaining nutrient demands for the test plants.

Eight experimental blocks (2.7 by 60 m) were established in an east-west direction. Within each block 16 plant rows were oriented lengthwise. North-south divisions were made to divide each block into 20 plots which became the experimental units. An entire plot was covered by an exposure chamber during treatment. Treatment plots measuring 2.4 × 2.4 m were selected within blocks on the basis of uniform plant size and distribution. Treatments were assigned to plots to minimize the possibility that a response trend would appear due to variability asociated with location. This was achieved within an experiment by assigning treatments with a common SO_2 concentration to the same block whenever possible and/or by assigning treatments which differed most to adjacent plots.

---

* The once-every-four-weeks treatment was repeated three times using slightly different starting dates.

The blocks were drilled on April 11, 1977 with wheat and barley at a rate of 100 kg seed/ha, alfalfa at 11 kg/ha, and crested wheatgrass at 9 kg/ha. All rows were drilled 18 cm apart. Sparse and spotty emergence of alfalfa required replanting of this test species on May 10, 1977. Poor development of crested wheatgrass resulting in its deletion from the field studies. One day after the second planting of alfalfa, experimental plots were sprayed with 2,4-DB amine (.6 kg/ha) to control broadleaf weeds. The herbicide was not applied to the alfalfa. Poor weed control resulted and weeding by hand was necessary. No additional cultural practices (e.g. irrigation) were performed.

Sulfur dioxide exposures were conducted in exposure chambers similar to those previously described by Heagle *et al.* (1972). The SO_2 was dispensed into exposure chambers and concentrations controlled as previously described (Heagle *et al.*, 1974). The portable exposure chambers covered the experimental plots only during actual SO_2 treatments. Ambient temperature (thermistors), humidity (Hygrodynamic Model 15-7012 Sensor), and sunlight (Solar-a-Meter Mark I-G100) measurements were made during the study period. Ambient SO_2 concentrations during the experimental period were always less than 1 pphm. Pollutant concentrations were continuously monitored during fumigations by drawing air from exposure chambers through Teflon sample lines to Meloy Labs sulfur analyzers (Model SA 285). The SO_2 analyzers were checked monthly for accuracy with a Monitor Labs Model 8500 Calibrator.

Visible injury to the foliage of the grain species was estimated three days after each fumigation and terminated in July due to the appearance of natural chlorosis and necrosis.

The grain crops were harvested on August 15. The 180 cm center sections of the two test rows per species were divided into two equal, 90 cm sections, providing 4 samples per treatment per species. The number of plants and seed heads per sample were counted. Grain heads were removed from each sample area, bagged, air dried and threshed with a Vogel wheat-head thresher. Kernels were oven dried at 50°C and weighed. Yields were expressed as gm of grain per plant.

Alfalfa was harvested on July 29, 1977 when at approximately 10% bloom. Sample areas were assigned as described for the grain species. Plants were clipped at soil level, oven dried at 70°C and weighed. Yield values were expressed as weight (gms) per plant.

Range Grass and Alfalfa Study

The objective of this study was to determine the effects of repeated SO_2 exposures during the vegetative stage of growth on the yield of alfalfa, *M. sativa* 'Ladak-65'; crested wheatgrass, *Agropyron desertorum* 'Nordan'; western wheatgrass, *Agropyron smithii*; Russian wild ryegrass, *Elymus junceus*; blue grama grass, *Bouteloua gracilis* and needle and thread grass, *Stipa comata*. Ladak-65 alfalfa, Nordan crested wheatgrass and Russian wild ryegrass were seeded and the remaining species were transplanted from cuttings on July 5, 1977. The growing medium was a mixture of three parts soil (sandy clay loam) to one part Jiffy-Mix-Plus. This mixture was amended with 2.3 kg of 11-33-11-5 (N, P, K, S) and 1.2 kg of hydrated lime per cubic meter of soil. Grasses were grown in 10 cm dia. by 30 cm high pots and alfalfa in 20 cm dia. by 18 cm high pots. Plants were thinned to one grass or two alfalfa plants per pot.

Treatments were 0, 25, 40, 80, or 120 pphm SO_2 repeated every 14 days between the hours of 8 and 11 a.m. The treatment plots were randomly arranged in an east-west direction and within plots, test species were grouped in rows oriented in an east-west direction. There were 13 to 14 pots per treatment of each grass species and 6 pots of alfalfa per treatment. All plots received five SO_2 exposures, separated by two weeks, beginning August 25. Test plants were covered by exposure chambers only during treatment with SO_2. Plants were watered as necessary to prevent moisture stress. Ambient environmental conditions and SO_2 concentrations were monitored as described in the earlier study. All plants were harvested November 3 while in the vegetative growth stage. Plant tops were clipped at soil level and roots were washed. Tops and roots were oven dried at 70°C and weighed.

Data Analysis

Preliminary examination of the data from the field exposure study indicated that a large block-to-block variation occurred, independent of treatment differences. Because of this variation, treatment comparisons were limited to those appearing in the same block. The effect of this limitation is variable and will be discussed with the analysis of the results for each experiment in the field exposure study.

The statistical treatment of the data involved analyses of variance and calculation of LSD's by Tukey's multiple comparison procedure (Snedecor and Cochran, 1967). The one exception was that in the multiple exposure experiment Bonferroni's multiple comparison method (Neter and Waserman, 1974) was used to compute significant t-values. The assumption inherent in the t-test and analysis of variance that the data be normally distributed with variances which do not increase with treatment means (Snedecor and Cochran, 1967) was tested and found to be true. Therefore, no corrective data transformations, such as the logarithmic transformation, were needed and the only modification of the data was to express yield on a weight per plant basis to adjust for differences in the number of plants between samples.

RESULTS

Average temperature, relative humidity, solar insolation and rainfall which occurred during the experimental periods are summarized in Table 15.1. Comparisons of desired and actual SO_2 concentrations for all experiments are presented in Table 15.2.

Field Exposure Study

Chronic Exposure Experiment

Comparatively severe limitations were imposed on this experiment by the large block-to-block variability which prevented comparisons of treatments located in different blocks. Two analyses were completed comparing 0 pphm, 3 pphm and 15 pphm treatments or the 5 and 10 pphm treatments. The block-by-block comparisons of the mean yields between SO_2 treatments are given in Table 15.3. The yields of Durum wheat and barley were suppressed 42 and 44%, respectively, comparing the mean at 0 to that at 15 pphm, and about 15% when comparing the mean yield at 5 pphm to the mean yield at 10 pphm. The differences, however, were not statistically significant at the 95% level of confidence. In the above comparisons, the mean yield at the highest SO_2 concentration was always the lowest, suggesting that the yield of Durum wheat and barley can be reduced by weekly, 72-hr exposures to SO_2 concentrations as low as 10 to 15 pphm.

Spring wheat seemed more resistant to the chronic SO_2 exposures. The mean of the 15 pphm treatment was actually greater by 7% than the 0 pphm treatment mean (control). Further, only a 5% yield difference occurred between the 5 pphm and 10 pphm treatments.

Foliar injury was observed on all species of small grains exposed to SO_2 in the field experiments. Symptoms of SO_2 injury appeared as chlorosis and necrosis on the leaf tips and margins. No similar injury was observed on plants not treated with SO_2. Foliar injury on small grains in the chronic exposure experiment was no greater than 15% at SO_2 concentrations of 3, 5, or 10 pphm (Table 15.4). At 15 pphm, the highest concentration used in this experiment, average injury values ranged from 25 to 50%.

Multiple Exposure Experiment

The block-to-block variability discussed earlier restricted comparisons of exposure frequencies to SO_2 concentrations completely contained within a block (25, 80 or 120 pphm).

Analyses of variance for Durum wheat, spring wheat, barley and alfalfa showed that 1) the pattern of differences in mean yields between exposure frequencies did not vary with SO_2 concentration (no significant exposure frequency by SO_2 concentration interaction) and 2) there were no significant differences in mean yields between different exposure frequencies at either 25, 80 or 120 pphm (Table 15.5 gives means and LSD's). Thus, varying the frequency of 3-hour exposures to SO_2 concentrations up to 120 pphm from as

TABLE 15.1. AVERAGE WEEKLY AMBIENT TEMPERATURE, HUMIDITY AND SOLAR INSOLATION OCCURRING DURING THE WEEKS OF EXPOSURE

	Average Ambient Environmental Conditions										
	Temperature (°C)					Relative Humidity (%)				Rainfall (cm)	
Week	Max.*/	Min.*/	Day†/ Avg.	Night‡/ Avg.	Solar Insolation†/ (cal./cm²/min)	Max.*/	Min.*/	Day†/ Avg.	Night‡/ Avg.		
5/6-5/12	18.1	-.4	13.4	3.8	.74	95	39	58	85	March	12.9
5/13-5/19	17.1	2.2	13.1	4.5	.62	96	42	58	87		
5/20-5/26	19.0	2.0	14.8	6.0	.68	97	37	56	87		
5/27-6/2	17.8	3.2	14.4	5.6	.64	96	45	63	91	April	2.6
6/3-6/9	25.1	9.0	20.8	11.9	.73	95	41	59	87		
6/10-6/16	24.8	5.2	19.6	9.1	.79	95	32	52	86		
6/17-6/23	26.3	6.8	21.2	11.0	.70	97	36	56	84	May	8.7
6/24-6/30	29.7	7.6	24.0	12.0	.89	89	22	34	73		
7/1-7/7	24.1	6.8	20.0	10.4	.73	88	24	36	68		
7/8-7/14	25.8	5.8	21.6	10.2	.74	93	26	39	76	June	2.9
7/15-7/21	28.2	7.0	22.6	11.3	.78	92	20	37	74		
7/22-7/28	30.3	9.6	24.6	13.7	.73	85	20	37	67		
7/29-8/4	35.6	11.0	30.0	16.0	.83	79	11	24	58	July	.3
8/5-8/11	37.2	11.9	30.5	17.7	.79	72	11	25	52		
8/12-8/18	35.9	8.7	28.6	16.4	.70	83	8	31	60		
8/19-8/25	24.5	10.9	20.0	13.3	.43	92	33	54	80	August	4.8
8/26-9/1	24.8	10.2	20.0	12.4	.58	93	22	43	83		
9/2-9/8	25.0	10.3	20.4	13.3	.54	90	24	44	78		
9/9-9/15	26.2	5.5	20.1	10.8	.56	91	14	35	69	September	9.1
9/16-9/22	18.1	6.9	13.6	9.3	.39	96	36	64	87		
9/23-9/29	17.3	5.7	13.0	8.0	.36	92	42	67	85		
9/30-10/6	17.9	3.8	12.5	7.4	.43	79	30	54	66	October	6.5
10/7-10/13	19.3	3.7	13.4	7.2	.43	88	31	54	76		
10/14-10/20	19.2	2.7	12.3	6.7	.38	93	34	62	77		
10/21-10/27	16.8	3.2	11.7	6.3	.32	97	51	73	91		

*/ Average of seven daily maximum hourly values.
†/ Weekly average of hourly values between 7 am and 8 pm.
‡/ Weekly average of hourly values between 8 pm and 7 am.

TABLE 15.2. COMPARISONS OF ACTUAL AND DESIRED SO_2 CONCENTRATIONS FOR ALL EXPERIMENTS

			Actual Concentration, pphm							
						% of time $\leq$ stated value*				
Study	No. Obs.	Desired Conc.	Min.	Avg.	Max.	5	10	50 (Median)	90	95
Multiple	192	25	9	24.0	80	14	17	25	27	30
	192	40	0	39.4	130	26	32	38	45	51
	192	80	29	74.6	110	46	56	78	83	84
	192	120	25	114.9	160	84	90	120	130	135
Growth	36	25	5	25.1	33	16	18	26	30	31
	36	40	0	36.5	47	0	30	38	44	44
	36	80	33	77.1	87	45	55	80	84	84
	36	120	25	112.8	135	25	85	123	130	130
Range Grass	30	25	18	25.2	35	19	22	25	27	28
	30	40	27	36.4	45	28	30	37	40	41
	30	80	38	86.1	120	45	46	90	105	120
	30	120	120	111.1	125	100	100	110	120	125
Chronic	864	3	0	3.0	9.0	1.8	2.0	2.7	4.0	5.6
	864	5	1.4	5.1	12.0	3.3	3.6	4.8	6.8	8.0
	864	10	0	9.7	20.0	6.6	7.5	9.5	12.0	14.0
	864	15	0	15.0	53.0	9.0	10.0	14.5	21.0	23.0

* For example, the first line indicates that 5% of the time the concentration was no more than 14 pphm, 10% of the time it was no more than 17 pphm, half the time it was no more than 25 pphm, 90% of the time it was no more than 27 pphm, 95% of the time it was no more than 30 pphm.

often as once every week to as infrequently as once every 5 weeks had no effect on yields of the test species.

A secondary objective of the multiple exposure experiment was to evaluate the effect of various SO_2 concentrations on yield. The lack of a significant effect on plant yield by varying the frequency of exposure was discussed above. Therefore, all plots which received the same SO_2 exposure concentration can be considered equivalent and pooled to assess the effect on yield of varying SO_2 concentrations. The results of pooling plots treated with a common SO_2 concentration and located in the same block are given in Table 15.6. No differences significant at the 95% level of confidence were found between SO_2 concentrations indicating the yields of Durum wheat, spring wheat, barley and alfalfa were independent of SO_2 concentration for these 3-hr duration exposures.

TABLE 15.3. YIELD RESPONSE OF SELECTED SMALL GRAINS TO WEEKLY, 72-HOUR EXPOSURES TO LOW SO_2 CONCENTRATIONS

BLOCK 1

	MEANS (gm/plant)[*]				
SPECIES	0 pphm	3 pphm	15 pphm	$\bar{X}_0 - \bar{X}_{15}$	LSD[‡]
Durum wheat	1.68	1.52	0.97	0.71 (42%)[†]	1.28
Spring wheat	1.34	1.81	1.44	-0.10 (7%)	1.67
Barley	1.49	1.64	0.83	0.66 (44%)	1.35

BLOCK 2

	MEANS (gm/plant)[*]			
	5 pphm	10 pphm	$\bar{X}_5 - \bar{X}_{10}$	LSD[‡]
Durum wheat	2.71	2.28	0.43 (16%)[†]	1.04
Spring wheat	1.85	1.76	0.09 (5%)	1.36
Barley	3.10	2.66	0.44 (14%)	1.10

[*] Means based on a sample size of four.

[†] Represents change relative to the smaller of the means.

[‡] Least significant difference (LSD) based on Tukey's studentized range procedure using error mean squares of .452 (Durum wheat), .760 (spring wheat) and .502 (barley) with 11 degrees of freedom.

TABLE 15.4. ESTIMATED PERCENT FOLIAR INJURY FOR SMALL GRAINS IN THE CHRONIC EXPOSURE EXPERIMENT

	Sulfur Dioxide concentration, pphm			
Species	3	5	10	15
Durum wheat	5%[*]	10%	15%	25%
Spring wheat	5	15	15	50
Barley	15	10	15	35

[*] Percent foliar injury readings made on June 30, 1977.

The foliar injury data are given in Table 15.7. No trends exist which would suggest that greater foliar injury occurred with more frequent SO_2 exposures; however, it is apparent that a positive correlation existed between foliar injury and SO_2 concentration. Averaged across species and frequency of exposure, foliar injury ranged from about 5% at 25 pphm SO_2 to about 35% at 120 pphm.

TABLE 15.5. EFFECTS OF VARYING FREQUENCY OF 3-HOUR SO_2 EXPOSURES ON YIELD OF SMALL GRAINS AND ALFALFA

SO_2 (pphm)	Wks between repeated exposures							$\bar{X}_M-\bar{X}_m$[†]	LSD[‡]
	1 (gm)	2 (gm)	3 (gm)	4a[*] (gm)	4b[*] (gm)	4c[*] (gm)	5 (gm)		
				DURUM WHEAT					
25	1.6[§]	1.1	1.3	1.2	1.4	1.1	1.3	0.5	1.7
80	3.8	3.8	2.5	3.5	3.8	2.6	3.6	0.3	1.7
120	2.2	2.2	2.2	1.9	2.2	2.7	1.6	1.1	1.7
				SPRING WHEAT					
25	2.0	1.4	1.8	1.8	1.2	1.6	1.8	0.8	2.2
80	2.7	3.1	2.2	2.5	3.0	3.1	3.4	1.2	2.2
120	3.0	2.5	2.1	2.2	2.0	2.1	2.3	1.0	2.2
				BARLEY					
25	1.9	2.0	1.8	1.9	1.9	1.8	2.3	0.5	1.8
80	2.4	3.8	2.4	3.7	2.7	3.1	2.5	1.4	1.8
120	1.8	2.5	2.7	2.2	2.2	2.8	1.9	1.0	1.8
				ALFALFA					
25	0.64	0.38	0.44	0.71	0.42	0.57	0.22	0.49	1.2
80	0.39	1.01	0.82	0.80	0.80	0.98	0.76	0.53	1.2
120	0.67	0.51	0.55	0.54	0.38	0.58	0.65	0.29	1.2

[*] Experiments started on different dates (May 13, May 19 or June 2).

[†] Maximum mean minus minimum mean.

[‡] LSD based on Tukey's studentized range procedure using error mean squares of .452 (Durum wheat), .760 (spring wheat), .520 (barley), and .225 (alfalfa) with 11 degrees of freedom.

[§] Means based on a sample size of four; means given in gm per plant.

Growth Stage Experiment

The block-to-block variability already discussed limited the analysis to comparisons of growth stages at SO_2 concentrations completely contained within one block (40, 80 and 120 pphm). Analyses of variance for Durum wheat, barley, spring wheat and alfalfa showed that 1) the pattern of differences in mean yields between growth stages did not vary with SO_2 concentration (no significant growth stage by SO_2 concentration) and 2) there were no significant differences in mean yield between growth stages at either 40, 80 or 120 pphm (see Table 15.8 for means and LSDs). Thus, when yields were averaged across the three SO_2 concentrations, the growth stage mean yields typically were within a range of plus or minus one standard error, indicating that the sensitivity of the test plants to SO_2 was not different with regard to growth stage.

TABLE 15.6. YIELD RESPONSE OF SELECTED SMALL GRAINS AND ALFALFA EXPOSED TO SO_2 FUMIGATIONS IN THE MULTIPLE EXPOSURE EXPERIMENT

BLOCK 3

	SO_2 (pphm)			
	0	120	$\bar{X}_{120}-\bar{X}_0$	t-value[§][§§]
Durum wheat	1.96[†]	2.12	0.16 (8%)[‡]	0.76
Spring wheat	2.41	2.31	-0.10 (4%)	-0.37
Barley	2.17	2.29	0.12 (6%)	0.53
Alfalfa	0.44	0.55	0.11 (25%)	0.74

BLOCK 4

	SO_2 (pphm)				t-values		
	0	40	80	$\bar{X}_{Max}-\bar{X}_{Min}$	40-0	80-0	80-40
Durum wheat	3.37	2.99	3.37	0.38 (13%)	-1.38	0.0	1.64
Spring wheat	2.28	2.39	2.86	0.58 (25%)	0.31	1.57	1.93
Barley	3.29	3.92	2.95	0.33 (11%)	0.88	0.29	-1.34
Alfalfa	0.39	0.96	0.77	0.37 (63%)	1.91	1.10	-1.16

BLOCK 5

	SO_2 (pphm)			
	25	40	$\bar{X}_{40}-\bar{X}_{25}$	t-value
Durum wheat	1.30	1.92	0.62 (48%)	2.94
Spring wheat	1.64	2.02	0.38 (23%)	1.39
Barley	1.92	2.07	0.15 (8%)	0.67
Alfalfa	0.48	0.41	-0.07 (17%)	-0.10

---

[*] No t-values significant at the 95% level of confidence.

[†] Average plant yield (grams dry weight of grain and foliage of small grains and alfalfa, respectively). Means were grouped over frequencies of exposures based on the following sample sizes: Block 3 (0 pphm-16; 120 pphm-28); Block 4 (0, 40 pphm-12; 80 pphm-28), Block 5 (25 pphm-28; 40 pphm-16).

[‡] Represents change relative to smaller of the means.

[§] Significance levels controlled simultaneously for group of 5 comparisons made per species.

[§§] Based on error mean squares of .452 (Durum wheat), .760 (spring wheat), .520 (barley) and .225 (alfalfa) with 11 degrees of freedom.

TABLE 15.7. ESTIMATED PERCENT FOLIAR INJURY FOR SELECTED SMALL GRAINS IN THE MULTIPLE EXPOSURE EXPERIMENT

Species	Desired Concentration (pphm)	Days Between Exposures				
		7	14	21	28[*]	35
Durum wheat	25	5%[†]	5%	5%	15%	5%
	40	5	15	15	5	10
	80	10	25	30	25	20
	120	20	30	40	45	30
Spring wheat	25	5	0	1	15	1
	40	5	15	1	15	10
	80	15	15	25	20	15
	120	25	30	35	30	40
Barley	25	1	1	5	10	1
	40	10	20	1	25	5
	80	25	15	30	40	20
	120	35	35	80	50	35

[*] Average of three 4th week regimes.

[†] Percent foliar injury readings made on or about July 1, 1977.

Range Grass and Alfalfa Study

The analyses of variance indicated that significant differences existed only for western wheatgrass roots and alfalfa tops (Table 15.9). However, the significance in each case was due to a single mean being greatly different than the others. For western wheatgrass, the 80 pphm mean was low, for alfalfa the 25 pphm mean was high. No systematic variation appeared among the SO_2 treatment means for any of the test species. The 25, 40, 80 and 120 pphm treatment means tended to vary haphazardly about the control (Figure 15.1). Among these four means, the minimum was typically less than 10% below the control and the maximum no more than 25% above.

Species by species comparison of the four treatment means to the control (Table 15.9) showed that 13 times the SO_2 means were less than the control means and 35 times they were greater. None of these differences were significant at the 95% level. Since standard errors were typically less than 10% of the means, the lack of systematic response to SO_2 could not have been due to excessive sampling variability. The results clearly indicate that under the conditions of biweekly, 3-hr exposures, SO_2 concentrations of up to 120 pphm had no effect on the growth of either the roots or tops of these six species.

TABLE 15.8. YIELD RESPONSE OF SELECTED SMALL GRAINS AND ALFALFA AT SEVERAL GROWTH STAGES TO SO_2 FUMIGATIONS

	Growth stage (biweekly) increments							
SO_2	1[†]	2	3	4	5	6	$\bar{X}_{Max}-\bar{X}_{Min}$	LSD[‡]
DURUM WHEAT								
40 pphm	1.7 gm	1.3 gm	1.6 gm	1.4 gm	1.9 gm	2.5 gm	1.2	1.7
80	1.2	1.6	2.0	1.4	1.5	1.7	0.4	1.7
120	2.2	2.5	2.0	2.9	2.5	2.3	0.9	1.7
SPRING WHEAT								
40	1.8	1.5	1.7	1.4	1.3	1.2	0.7	2.2
80	1.4	2.0	2.1	1.5	1.6	1.8	0.7	2.2
120	2.1	2.4	1.4	1.9	1.4	2.3	1.0	2.2
BARLEY								
40	2.8	2.6	2.3	2.4	3.1	2.0	1.1	1.8
80	1.2	1.7	1.8	1.6	1.3	1.1	0.7	1.8
120	3.4	4.9	3.5	2.2	3.2	3.7	2.7*	1.8
ALFALFA								
40	0.36	0.77	0.57	0.50	0.45	0.86	0.50	1.2
80	0.49	0.40	0.52	0.51	1.14	0.36	0.78	1.2
120	0.57	1.34	0.61	0.98	0.52	0.64	0.82	1.2

*Significant at 95% level of confidence.

[†] Average plant yield in (grams dry weight of grain and foliage of small grains and alfalfa, respectively) based on a sample size of four.

[‡] LSD based on Tukey's studentized range procedure using error mean squares of .452 (Durum wheat), .760 (spring wheat), .520 (barley) and .225 (alfalfa) with 11 degrees of freedom.

DISCUSSION

Weekly exposure of Durum wheat and barley for 72 hr at an SO_2 concentration of 15 pphm suppressed average grain yields 42 and 44%, respectively, compared with the 0 pphm treatment. This suppressive effect was also observed as the SO_2 concentration increased from 5 to 10 pphm, but the effect was considerably less. Though these yields differences were not significant at the 95% level of confidence, it is probably that the lack of statistical significance between the treatment means for Durum wheat and barley was due to 1) the small sample size which was related to experiment limitations (preventing treatment replication) or 2) the inability to make block-to-block comparisons.

A similar yield suppression was not observed with spring wheat indicating that this species was more resistant. Compared to Durum wheat and barley, the

TABLE 15.9. YIELD RESPONSE OF RANGE GRASS SPECIES AND ALFALFA TO SO_2 FUMIGATIONS.

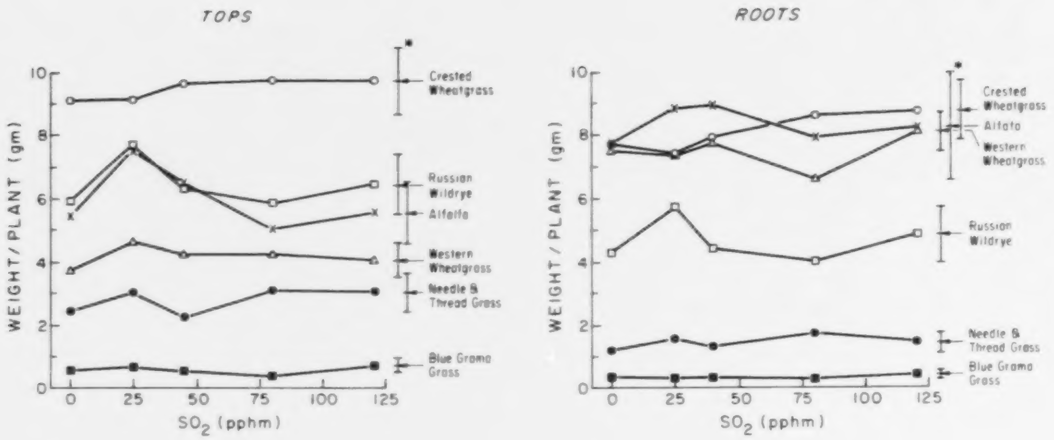
	TREATMENT MEANS†/								
SPECIES	0 pphm	25 pphm	40 pphm	80 pphm	120 pphm	$\bar{X}_M-\bar{X}_m$‡/	LSD	SE§/	F
Crested wheatgrass									
Tops	9.1 gm	9.1 gm	9.6 gm	9.7 gm	9.7 gm	0.6	2.1	0.53	0.38
Roots	7.7	7.4	7.9	8.6	8.7	1.3	1.8	0.46	1.62
Western wheatgrass									
Tops	3.7	4.6	4.2	4.2	4.0	0.9	1.1	0.28	1.40
Roots	7.5	7.3	7.7	6.5	8.0	1.5*	1.2	0.31	3.30*
Russian wildrye									
Tops	5.9	7.7	6.3	5.8	6.4	1.9	2.0	0.49	2.42
Roots	4.3	5.7	4.4	4.0	4.8	1.7	1.8	0.43	2.24
Blue grama grass									
Tops	0.58	0.63	0.58	0.39	0.69	0.30	0.48	0.12	0.86
Roots	0.34	0.30	0.31	0.23	0.40	0.17	0.31	0.08	0.68
Needle & Thread Grass									
Tops	2.4	3.0	2.2	3.1	3.0	0.9	1.3	0.33	1.50
Roots	1.2	1.6	1.3	1.7	1.4	0.5	0.7	0.17	0.46
Alfalfa									
Tops	5.4	7.5	6.5	5.0	5.5	2.5*	2.1	0.50	3.88*
Roots	7.7	8.8	8.9	7.9	8.2	1.2	3.5	0.85	0.40

*Significant at 95% confidence level.

†/Means based on sample sizes of 14 (crested wheatgrass, western wheatgrass), 13 (Russian wildrye, blue grama grass, needle and thread grass) and 6 (alfalfa); grams per plant.

‡/Maximum mean minus minimum mean.

§/Standard error.



*Each bar equals 2 standard errors.

*Each bar equals 2 standard errors.

Figure 15.1. Yield response of tops and growth response of roots of range grass species and alfalfa to various SO_2 concentrations.

foliage of spring wheat was severely injured, but the yield of this species appeared to be less affected by the SO_2 treatments. This, along with results of associated studies discussed below, suggests that within the environmental conditions of this research, small grain species can sustain significant foliar injury without accompanying yield suppression. Based on these observations, foliar injury is not a reliable predictor for estimating grain yield losses.

There was no stage of growth during which the yield of Durum wheat, spring wheat, barley or alfalfa was affected most by a single, 3-hr exposure to SO_2 concentrations up to 120 pphm. These treatments repeated as frequently as once a week also failed to affect yields. The average foliar injury to the small grain species exposed to 120 pphm was approximately 35%. Apparently, sufficient intervals of time were present between repeated exposures to allow physiological recovery and a decrease in yields did not occur. This recovery phenomenon was described by Zahn (1963), when he observed that plant yields were less affected by SO_2 as the period of time between successive exposures was increased.

The small grain species and alfalfa were tolerant of the relatively short, 3-hr SO_2 exposures when treated under the environmental conditions which occurred during this investigation. This tolerance to SO_2 could be related to the low rainfall levels which occurred in June and July (Table 15.1). Although soil moisture was not measured within the experimental plots, the presence of low moisture values can be inferred from the low levels of precipitation during these months. It was also during this period of time that grain heads were developing. Wells (1915), van Haut (1961) and van Haut and Stratmann (1970), found that the heading-out stage is one of the critical phases of growth during which exposure of grain crops to SO_2 would be most likely to suppress yields. Therefore, the absence of adequate soil moisture may have increased the tolerance of the crops to SO_2 during this critical growth stage. This aspect of the experimental studies can be related to expected effects on non-irrigated land within the Poplar River Valley of Montana, since average moisture conditions are typically low in this region during the time when grain heads are developing. This ambient condition could increase the tolerance of plants to SO_2, similar to that suspected in this research. The yields of small grains in this study were similar to average yields for grain grown in non-irrigated areas of northeastern Montana (Bowman and Shaw, 1976, 1977) which typically experience low rainfalll during the later growth stages.

Biweekly exposures (every 14 days) of five range grasses and alfalfa for periods of 3 hr at concentrations of up to 120 pphm SO_2 did not affect the growth of roots or yield of tops. The range grasses failed to develop in field plot studies, and cuttings transplanted from greenhouse cultures developed slowly. As a result, exposures to SO_2 were not started until late summer and were, therefore, associated with cool temperatures (Table 15.1). The exact cause of the lack of response of the range grasses and alfalfa to the SO_2 treatments is difficult to assess, but two possibilities are apparent: (1) low temperatures during treatment and (2) inherent resistance to SO_2. Cool temperatures which prevailed during this study tend to reduce plant response to SO_2 (Heck *et al.*, 1965; Guderian, 1977). Other investigators found that the injury (Tingey *et al.*, 1978) and growth (Dodd, Section 13 of this report) responses of several plant species used in this study were not significantly affected by a single SO_2 exposure to 100 pphm for 4 hours or by seasonal median SO_2 concentrations up to 6.8 pphm (concentrations were lognormally distributed with a standard geometric deviation of approximately 2.5), respectively. A reasonable analysis of the response of the range grasses and alfalfa used in this study is that they appear to be relatively tolerant (based on foliar injury and growth response) to SO_2 exposures. A similar analysis, however, apparently does not exist with regard to another response parameter—nutritive quality. An analysis of the nutritive quality of western wheatgrass and prairie June grass demonstrated that seasonal median SO_2 concentrations up to 6.8 pphm during two growing seasons reduced crude protein content and digestibility of western wheatgrass (Schwartz *et al.*, 1978).

CONCLUSIONS

The yields of Durum wheat and barley treated weekly with 15 pphm SO_2 for 72 continuous hr were 42 and 44% of the control, respectively. These results were not significant at the 95% probability level, but the data suggested that weekly, 72-hr exposures to SO_2 concentrations as low as 10-15 pphm could suppress yields. The yield of spring wheat was not affected by a similar treatment. Varying the frequency of 3-hr exposure to SO_2 concentrations up to 120 pphm from as often as once every week to as infrequently as once every 5 weeks had no effect on yields of the small grains or alfalfa. No growth stage was identified during which the small grains or alfalfa were most sensitive to a single 3-hr exposure to SO_2 concentrations up to 120 pphm. The growth of tops and roots of range grasses and alfalfa were not affected by 3-hr exposures to concentrations of SO_2 up to 120 pphm repeated at 14-day intervals during September and October.

ACKNOWLEDGEMENTS

The authors acknowledge the capable assistance of the following Oregon State University Crop Science Department technical personnel in the conduct of research described in this report: Alex March, Sharon Newton, Milton Plocher and Rick Voelkel. The excellent supervision of employees and coordination of research activities by Mr. Shelton Perrigan was greatly appreciated. The skill of Nancy Lanpheare, the typist, greatly facilitated the preparation of this manuscript.

This research was funded jointly by EPA Region VIII and the Corvallis Environmental Research Laboratory. George Bolter, the Region VIII coordinator for this project, assisted in the design of the experiment.

REFERENCES

Bennett, J. H. and A. C. Hill. 1973. Inhibition of Apparent Photosynthesis by Air Pollutants. J. of Envir. Quality, 2(4):526-530.

Booth, J. A., G. O. Thorneberry and M. Lujan. 1976. Crop Reactions to Sulfur Dioxide in New Mexico. University Park, New Mexico State Univ. Agric. Experiment Sta. Bull. 645, 27 pp.

Bowman, H. and A. F. Shaw. 1977. Performance Summary, Barley. Bull. 1094. Cooperative Extension Service, Montana State University, Bozeman. 21 pp.

Bowman, H. and A. F. Shaw. 1976. Performance Summary, Spring Wheat Varieties. Bull. 1098. Cooperative Extension Service, Montana State University, Bozeman. 30 pp.

Brough, A. and M. A. Parry. 1976. Effects of Aerial Pollutants on Cereal Growth. Rothamsted Exp. Sta. Report for 1975, Part 1:41-42.

Davis, C. R., D. R. Howell and G. W. Morgan. 1966. Sulfur Dioxide Fumigations of Range Grasses Native to Southeastern Arizona. J. Range Management, 19(2):60-64.

Guderian, R. 1977. Air pollution. Springer Verlag, Berlin. 127 pp.

Heagle, A. S., D. E. Body and E. K. Pounds. 1972. Effect of Ozone on Yield of Sweet Corn. Phytopathology, 62:683-687.

Heagle, A. S., D.E. Body and G. E. Neely. 1974. Injury and Yield Responses of Soybean to Chronic Doses of Ozone and Sulfur Dioxide in the Field. Phytopathology, 64:132-136.

Heck, W. W., J. A. Dunning, and I. J. Hindawi. 1965. Interactions of Environmental Factors on the Sensitivity of Plants to Air Pollution. J. Air Pollut. Cont. Assoc.,15:511-515.

Heitschmidt, R. K. 1977. Chronic Effects of SO_2 Western Wheatgrass in a Montana Grassland. Ph.D. Thesis. Colorado State Univ., Fort Collins, Colorado. 100 pp.

Hill, A. C., S. Hill, C. Lamb and T. W. Barret. 1974. Sensitivity of Native Desert Vegetation to SO_2 and to SO_2 and NO_2 Combined. J. Air Pollut. Control. Assoc., 24:153-157.

Lamb, C. 1972. Sensitivity to Sulfur Dioxide Injury of Plant Species Native to the Southwest Desert Region. M.S. Thesis. Dept. of Biology, University of Utah, Salt Lake City. 62 pp.

Neely, G. E., D. T. Tingey, and R. G. Wilhour. 1977. "Effects of Ozone and Sulfur Dioxide Singly and in Combination on Yield, Quality and N-Fixation of Alfalfa," Proceedings of Internatinal Conference on Photochemical Oxidant Pollution and Its Control, EPA-600/3-77-001b, 2:663-673.

Neter, J. and W. Wasserman. 1974. Applied Linear Statistical Models. Richard D. Irwin, Inc., Homewood, Illinois. 842 pp.

Rabe, R. and Kreeb, K. 1976. Bioindikation bei Luftverunreinigungen Durch Messung der Aktivitat Verschiedener Enzyme von Testpflanze. (Bioindication of Air Pollution by Measuring the Activity of Various Enzymes of Test Plants.) Vortr Tag Umweltforsch Univ Honenheim 1976:73-78.

Schwartz, C. C., W. W. Lauenroth, R. K. Heitschmidt and L. L. Dodd. Manuscript has been submitted to and accepted, pending minor revision, by Journal of Applied Ecology for publication in fall, 1978.

Snedecor, G. W. and W. G. Cochran. 1967. Statistical Methods, 6th ed. Iowa State University Press, Ames, Iowa. 592 pp.

Tingey, D. T., L. Bard and R. W. Field. 1978. The Relative Sensitivity of Selected Plant Species to Several Pollutants Singly and in Combination. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Third Interim Report, Colstrip, Montana, December, 1977, E. M. Preston and R. A. Lewis, eds. EPA-600/3-78-02, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 508-513.

van Haut, H. 1961. Die Analyse von Schwefeldioxidwirkungen auf Pflanzen im Laboratoriumversuch. Staub. 21:52-56.

van Haut, H. and H. Stratmann. 1970. Farbtafelatlas über Schwefeldioxidwirkugen an Pflanzen. Essen, W. Germany Verlag W. Giradet. 206 pp.

Wells, A. E. 1915. Fumigation Experiments to Determine the Effect of Highly Diluted Sulfur Dioxide on a Growing Grain Crop. Bull. 98. U.S. Bur. Mines (Rep. Selby Smelter Comm.):213-307.

Zahn, R. 1963. Ueber den Einfluss Verschiedener Unweltfaktoren auf die Pflanzenempfindlichkeit Gegeneuber Schwefeldioxyd. Z. Pflanzenkr. Pflanzekesch. 70:81-95.

SECTION 16

PLANT COMMUNITY CHANGES DUE TO LOW LEVEL SO_2 EXPOSURE

J. E. Taylor and W. C. Leininger

ABSTRACT

Changes in plant community structure due to artificial air pollution stressing were monitored by studying canopy cover, species diversity and phenology. In addition, aerial and ground photography was accomplished at periodic intervals during the growing season. Vegetation on the two ZAPS sites is responding differently to SO_2 fumigation. This is shown in differential responses of individual species and of graminoids versus forbs. These changes are partly confounded with inherent site differences between and within the study areas. Species diversity is not decreasing with SO_2 fumigation, whether based on canopy cover or number. Evenness and species richness are about equally influential on both ZAPS sites. Number-based diversity values are more variable than indices derived from cover because of the strong influence on evenness of sporadic flushes of high-number, low-cover annual species. Phenology data have not reflected treatment differences. Significant changes in lichen cover have occurred over the years. Though never abundant, lichen cover has been strikingly depressed by SO_2 fumigation. This is more strongly observed on ZAPS II, where growing conditions are less favorable and fumigation is more intense. Preliminary stereoscopic analysis of ground photo plots suggests this procedure can yield accurate estimates of cover and number, but some problems remain to be resolved.

INTRODUCTION

The background and objectives of our overall project are given in the Introduction to Section 3. Research on the Zonal Air Pollution Study (ZAPS) site near Ft. Howes, is discussed in this section. This research consists of two interrelated but functionally independent parts: ground studies and aerial photography. The division is somewhat arbitrary because part of the data from ground studies is used as ground truth for the aerial photography. Thus, a portion of the data discussed here also is used in connection with aerial monitoring in Section 24.

The research to be discussed in this section includes studies of canopy coverage, species diversity, plant phenology, and ground-level photography. These are organized in the two general topics, Plant Community Analysis and Photographic Monitoring.

MATERIALS AND METHODS

Methodological details are presented in Section 3. The following discussion is restricted to the modification of those details on the ZAPS sites.

Plant Community Analysis

Canopy Coverage

Data were collected on the ZAPS sites at four times during the 1977 growing season: 21-22 May, 26-27 June, 26-27 July, and 4-5 September. These dates approximated the stages of early growth, peak of cool-season green, peak of warm-season green, and summer dormancy, respectively.

Each of the ZAPS plots was arbitrarily subdivided for sampling, with equal observations collected from the upper (north) and lower (south) half. These were handled as "replicates" in the statistical analyses, although they were not randomly designated; the lower area was always termed the first replicate, and the upper the second.

Two restrictions were imposed on sample plot positioning. Microtopographic depressions (run-in moisture areas) were avoided and no plots were placed less than one meter from a gas delivery pipe.

Diversity

As at the Colstrip locations, both density and cover data were collected concurrently at every sampling date. Diversity, evenness, and species richness were calculated from each data type. Species encountered in the samples, as well as others observed on the sites, are given in Appendix 16.1.

In several analyses, linear regression procedures were used to illustrate relationships between SO_2 levels and plant responses. The fumigation rates used were the theoretical ones of 0, 2, 5, and 10pphm for control, low, medium, and high, respectively. The actual values varied slightly (Sections 9 and 10).

Phenology

The phenological stages of all recognizable species were coded at each of six observation dates in 1977.

Lichens

During the 1977 cover sampling we noticed the striking absence of lichens from the plots receiving the higher rates of SO_2. Eversman (1977) reported similar findings with lichens which she had transplanted into the ZAPS plots. This prompted us to review our data from previous years to see if temporal changes had indeed occurred. Because of the low initial abundance of lichens in the area and their characteristically clumped distribution patterns, we analyzed lichen cover over all samples within years. As in other canopy data, we used five contiguous frames as one sampling unit. All these techniques served to reduce sample variability. Lichen numbers were not estimated because of the difficulty in distinguishing individual plant units *in situ*.

Photographic Monitoring

We used our standard ground and aerial photography procedures as described in Section 3 and 24. Aerial photo missions were timed to coincide with ground samples.

RESULTS AND DISCUSSION

Plant Community Analysis

Canopy Coverage

A summary of coverage data for ZAPS I and II is given in Tables 16.1. and 16.2., respectively. Means of vegetational categories are presented in Figure 16.1.

The treatment responses on the two ZAPS sites are difficult to compare because of pretreatment differences in species composition. Variations in soils, climate, and recent grazing histories all contribute to these differences.

In ZAPS I there is a tendency for a decrease in grasses and a corresponding increase in forbs with increasing fumigation. Total vegetation shows no consistent pattern. Lichens show a general decrease across treatments. Reverse trends are observed in ZAPS II, at least for grasses and forbs. Lichens, while never abundant, are essentially absent on the higher treatments of SO_2.

TABLE 16.1. CANOPY COVERAGE (PERCENTAGE) FOR ZAPS I, 1977.

SPECIES	21 MAY				26 JUNE				26-27 JULY				4 SEPT			
	CONT.	LOW	MED.	HIGH	CONT.	LOW	MED.	HIGH	CONT.	LOW	MED.	HIGH	CONT.	LOW	MED.	HIGH
GRAMINOIDS																
Agropyron smithii	21.81	10.75	13.81	17.81	26.75	15.88	14.19	19.69	19.94	20.88	17.44	19.38	19.20	15.15	16.15	24.90
Aristida longiseta	.81	4.50	1.13	2.94	4.25	4.13	.75	2.38	2.13	6.00	2.56	7.63	1.45	5.85	4.25	2.25
Bouteloua gracilis	.44	2.94	.38	.06	.38		.38	.38		1.00	1.38	.38		.05	.05	1.60
Bromus sp.*	2.00	.75	1.88	1.31												
B. japonicus					4.25	1.06	1.56	1.13	3.50	.69	1.31	.94	5.80	.50	1.00	1.90
Calamagrostis montanensis	1.06	.25	.31		.56	.31	.50		.06	.13				.05		
Carex pennsylvanica			.38	.44				.38				.44				.90
Danthonia unispicata	.38															
Festuca idahoensis			.38		2.00											
Juncus interior					.06											
Koeleria cristata	16.19	12.50	11.50	9.06	22.31	19.81	23.75	8.06	17.00	12.50	18.66	9.88	15.65	17.60	21.85	9.15
Muhlenbergia cuspidata																
Poa pratensis	4.06	2.13	2.94	.25		1.75	3.94	.13		.63	1.25	1.38	1.05	.70	.40	.65
P. sandbergii	3.31	4.50	4.25	4.69	5.44	2.88	2.25	5.63	6.06	5.81	7.44	4.31	3.50	1.85	2.30	6.15
Schedonnardus paniculatus								.44							.05	
Sporobolus cryptandrus										1.31						
Stipa comata	3.44	10.63	3.75	.81		11.50	3.75	2.00	6.94	15.81	6.94	.06	.35	4.30	7.35	.05
S. viridula	2.19		.06	.38	2.69			.38	.50			.06	2.55			.05
FORBS																
Achillea millefolium	8.31	2.00	8.88	16.31	15.38	2.25	5.75	20.19	9.13	1.06	8.63	15.63	7.45	1.46	3.85	15.95
Agoseris glauca				.69												
Ambrosia psilostachya																.05
Androsace occidentalis			.06													
Antennaria sp.		.25	.94													
A. neglecta	.25	.13	.50		.56	2.31	5.75	2.25		.50						
A. rosea		2.00	1.60	.44						.06	3.25	1.31	3.50	4.65	2.80	1.50
Arnica sororia				.75												.65
Aster falcatus										.94						
Astragalus sp.									.06			.38		.05		
A. crassicarpus		.38		.38	1.31		.44		.38							
A. purshii									.06	.38						
Bahia oppositifolia										.06						
Cerastium arvense										.13						
Conyza canadensis					.06			.06								
Erigeron divergens	.44	.06	.38	.38	.38	2.00	1.19	2.56	.44	2.81		2.06		1.96	1.25	2.40
Gaura coccinea					.06	.06										
Grindelia squarrosa					.75					.38	.38		.30			.30

* Includes *B. japonicus* and *B. tectorum* which were indistinguishable early in the season.

TABLE 16.1. (continued)

SPECIES	21 MAY				26 JUNE				26-27 JULY				4 SEPT			
	CONT.	LOW	MED.	HIGH	CONT.	LOW	MED.	HIGH	CONT.	LOW	MED.	HIGH	CONT.	LOW	MED.	HIGH
FORBS (continued)																
Hedeoma hispida	.13			.06	.31								.20			
Lepidium densiflorum	.13															
Leucocrinum montanum								.38								
Lomatium sp.				.50									.10			.05
Lupinus sp.			.25													
Mammillaria missouriensis															.05	
Melilotus officinalis								.50				.06				
Opuntia fragilis						.06					.06		.05			
Orthocarpus luteus	.38		.38	.38	.50		.56	6.56			1.19	3.75	.15	.05	.20	3.05
Petalostemon purpureum										.94						
Phlox hoodii											.06					
Plantago sp.*	.75	.88	.63	.50												
P. patagonica					.25	.75	.69	.31	.25	.81	.38	.44	.35	.70	.10	.55
P. spinulosa					.44			.44	.44	.06			2.35			.20
Polygonum viviparum					.25			.25	.06	.13	.19	.13	.80	.05		.05
Psoralea argophylla	.31					.44		.44				.44		.45	.05	.35
Solidago missouriensis												.06				
Sphaeralcea coccinea	.75	.38	.25	1.50	1.75	1.44	6.06	4.63	1.50	.56	4.81	1.69	1.35	2.80	1.10	.75
Taraxacum officinale	9.88	1.25	12.56	12.69	10.00	4.25	13.19	12.63	3.31	4.13	12.00	8.13	1.85	1.45	4.05	5.45
Tragopogon dubius	7.94	5.69	6.94	7.56	10.19	11.50	10.81	9.00	9.25	6.44	8.00	8.88	4.20	7.60	9.15	4.60
Vicia americana	.44			.06								1.31				
Zygadenus venenosus		.06	.63	.13												
Misc. forbs	.50		.12	.32	.88	.44			.19	.13			.25	.50		
HALF-SHRUBS AND SHRUBS																
Artemisia frigida	.38		1.38	.88			.06	1.50			.13	1.25			.30	1.35
A. tridentata					.06											
OTHERS																
Bare ground	2.94	3.75	8.69	3.38	4.38	3.19	3.63	4.25	5.25	5.56	5.63	4.00	5.30	6.35	5.20	4.00
Lichen	4.88	1.50	1.50	1.25	6.75	1.94	8.44	5.56	4.94	3.19	5.25	1.69	11.80	4.00	2.35	1.15
Moss	3.25	11.50	2.25	1.00	3.75	5.50	3.50	7.06	4.69	9.44	.88	1.13	4.55	10.30	.30	2.45
Litter	60.63	59.50	67.56	74.56	72.38	77.50	67.19	61.06	74.06	73.88	76.44	81.56	70.40	71.10	81.85	80.30
Rock		.06			.13			.19	.19	.25	.06					.05
TOTAL VEGETATION	94.39	74.90	79.38	83.44	122.31	90.25	107.50	114.87	90.95	96.69	101.57	92.76	88.80	91.95	78.95	88.45
TOTAL GRAMINOIDS	55.69	48.94	40.75	37.31	68.69	57.31	51.06	40.56	56.13	64.75	56.38	44.44	49.55	56.05	53.40	47.60
TOTAL FORBS	30.19	13.00	33.50	42.63	43.06	25.50	44.44	60.19	25.25	19.31	38.94	44.25	22.90	21.60	22.60	35.90
TOTAL SHRUBS	.38		1.38	1.25	.06		.06	1.50			.13	1.25			.30	1.35

* Includes both *P. Patagonia* and *P. spinulosa* which were indistinguishable early in the season.

TABLE 16.2. CANOPY COVERAGE (PERCENTAGE FOR ZAPS II, 1977.)

SPECIES	22 MAY				27 JUNE				27-28 JULY				5 SEPT			
	CONT.	LOW	MED.	HIGH	CONT.	LOW	MED.	HIGH	CONT.	LOW	MED.	HIGH	CONT.	LOW	MED.	HIGH
GRAMINOIDS																
Agropyron smithii	34.94	30.75	17.19	14.19	40.31	41.00	25.69	37.63	34.19	41.31	25.88	32.81	36.85	42.95	29.30	31.30
Aristida longiseta												.38				
Bouteloua gracilis			.13	.50	.06		4.25	.06		.94	1.94	2.69	.10	1.70	3.35	
Bromus sp.*	9.94	3.56	7.69	1.81												
B. japonicus					9.00	5.56	12.25	4.81	12.81	6.75	9.19	8.81	9.75	7.10	7.10	4.75
B. tectorum					.44	.31		.25	.06	.44		.06	.10		.30	
Carex filifolia								.94								
Koeleria cristata	.50	.06	11.13	29.50	9.81	.13	11.63	23.25	7.38		9.88	16.06	1.35	.05	11.00	15.45
Muhlenbergia cuspidata											.38					
Poa pratensis		.50	1.75	.69	.75	6.56	6.25	.13	.94	5.19	9.56		.10	1.65	2.75	.05
P. sandbergii	10.56	11.81	7.50	5.13	8.88	10.06	5.75	6.50	8.44	6.25	5.06	5.25	6.05	5.20	3.30	2.55
Schedonnardus paniculatus		.06			.63	.88	.19	.38	.88	.13	.25	.06	.55	.10		.15
Stipa comata								1.25			.75	1.69			.05	.95
S. viridula	.06	.94	13.00	10.94	2.06	2.81	22.81	19.94		2.19	16.06	14.19		.40	6.85	10.30
FORBS																
Achillea millefolium	2.00	2.25	.38		3.50	3.88	1.31	.06	2.25	2.44	3.81	.44	.30	2.20	.95	.05
Androsace occidentalis	.69	.31	.06	.06	.19			.19	.13			.19				
Antennaria neglecta										.06						
Astragalus sp.			.38													
A. drummondii							.06									
A. purshii											.50					
Bahia oppositifolia			.75	.25			2.75	5.31	1.63	.06	1.75	3.31			2.00	1.15
Camelina microcarpa	.06		.06													
Conyza canadensis	.06	.13														
Erigeron divergens	.06					.38	.13			.38					.10	
Hedeoma hispida	.44	.06			.13		.63	.25	.31		.38	.31	.25		.05	.05
Heterotheca villosa		.06														
Lappula echinata	.06		.06													
Lepidium densiflorum	10.94	1.31	1.81	2.25	6.44	1.35	3.31	3.31	4.75	1.50	1.50	4.75	10.75	.85	1.10	1.80
Leucocrinum montanun				.38												
Lomatium sp.	.31	.06		.81				.06								
Mammillaria missouriensis			.06		.06					.06						
Melilotus officinalis									.44	.50						
Opuntia fragilis		.06		.06	.06	.06	.38							.30	.30	
O. polyacantha	.38			.13												
Orthocarpus luteus	.13			.06							.06				1.70	

* Includes *B. japonicus* and *B. tectorum*, which were indistinguishable early in the season.

TABLE 16.2. (continued)

SPECIES	22 MAY				27 JUNE				27-28 JULY				5 SEPT			
	CONT.	LOW	MED.	HIGH	CONT.	LOW	MED.	HIGH	CONT.	LOW	MED.	HIGH	CONT.	LOW	MED.	HIGH
FORBS (continued)																
Phlox hoodii	1.94	.50	1.25	1.63	2.44	.06	3.06	2.00	4.19		1.94	2.56	2.25	.75	2.40	1.60
Plantago sp.*	.31	.13		.06												
P. patagonica														.05		
P. spinulosa					.88	.25	.13	.31	.69	.06	.13	.06	.35	.25	.10	.05
Polygonum viviparum														.35		
Sphaeralcea coccinea	.63	1.06	.88		1.44	2.13	.63	.81	.19	.13	1.31	.63	.10	.05	.25	.75
Taraxacum officinale	13.19	29.00	14.81	2.81	20.38	43.88	9.94	2.38	8.38	26.88	16.75	3.44	5.85	1.75	5.80	.30
Tragopogon dubius	1.69	2.31	4.56	1.69	6.38	7.31	6.25	5.75	2.38	1.44	5.06	2.31	2.70	1.90	1.90	.75
Vicia americana	2.13	1.44	5.13	5.56	.81	3.56	9.25	7.63	.25	.06	3.56	.88	.10	.05	.50	.15
Viola nuttallii		.13														
Misc. forbs		.19	.25	.13					.06							
HALF-SHRUBS AND SHRUBS																
Artemisia cana												.06				
A. frigida	.06		.69	.06	.94		1.50		1.44	.38	1.00	1.63	2.85	.40	1.60	.40
A. tridentata																1.30
Ceratoides lanata				.88				3.75				1.63				
OTHERS																
Bare ground	8.38	9.88	5.75	9.88	8.44	4.19	6.81	13.88	8.00	4.63	8.19	5.94	6.95	5.40	10.90	8.15
Lichen	2.63	2.25	.06		3.94	1.94	.75	.19	4.69	1.50	.31		2.55	1.40	.50	.15
Moss	3.31	1.69	.31		3.06	1.56	.13		.19	.25	.25		.05	.20	.10	
Litter	65.56	62.19	61.38	57.06	65.50	77.60	77.13	70.25	79.81	83.31	81.00	83.31	78.95	82.10	75.30	80.30
Rock								.50	.13		.06	.06	.10	.10	.15	.10
TOTAL VEGETATION	97.00	90.63	89.87	79.56	123.13	133.81	130.89	122.13	96.63	98.88	117.25	104.19	92.90	90.65	82.35	74.00
TOTAL GRAMINOIDS	56.00	47.69	58.38	62.75	71.94	67.31	88.81	90.13	64.69	63.19	78.94	82.00	64.85	59.15	63.00	65.50
TOTAL FORBS	35.00	39.00	30.44	15.88	43.25	63.00	39.81	28.06	25.63	33.56	36.75	18.88	22.60	29.50	17.15	6.65
TOTAL SHRUBS	.06		.69	.94	.94		1.50	3.75	1.44	.38	1.00	3.31	2.85	.40	1.60	1.70

---

* Includes *P. patagonica* and *R. spinulosa*, which were indistinguishable early in the season.

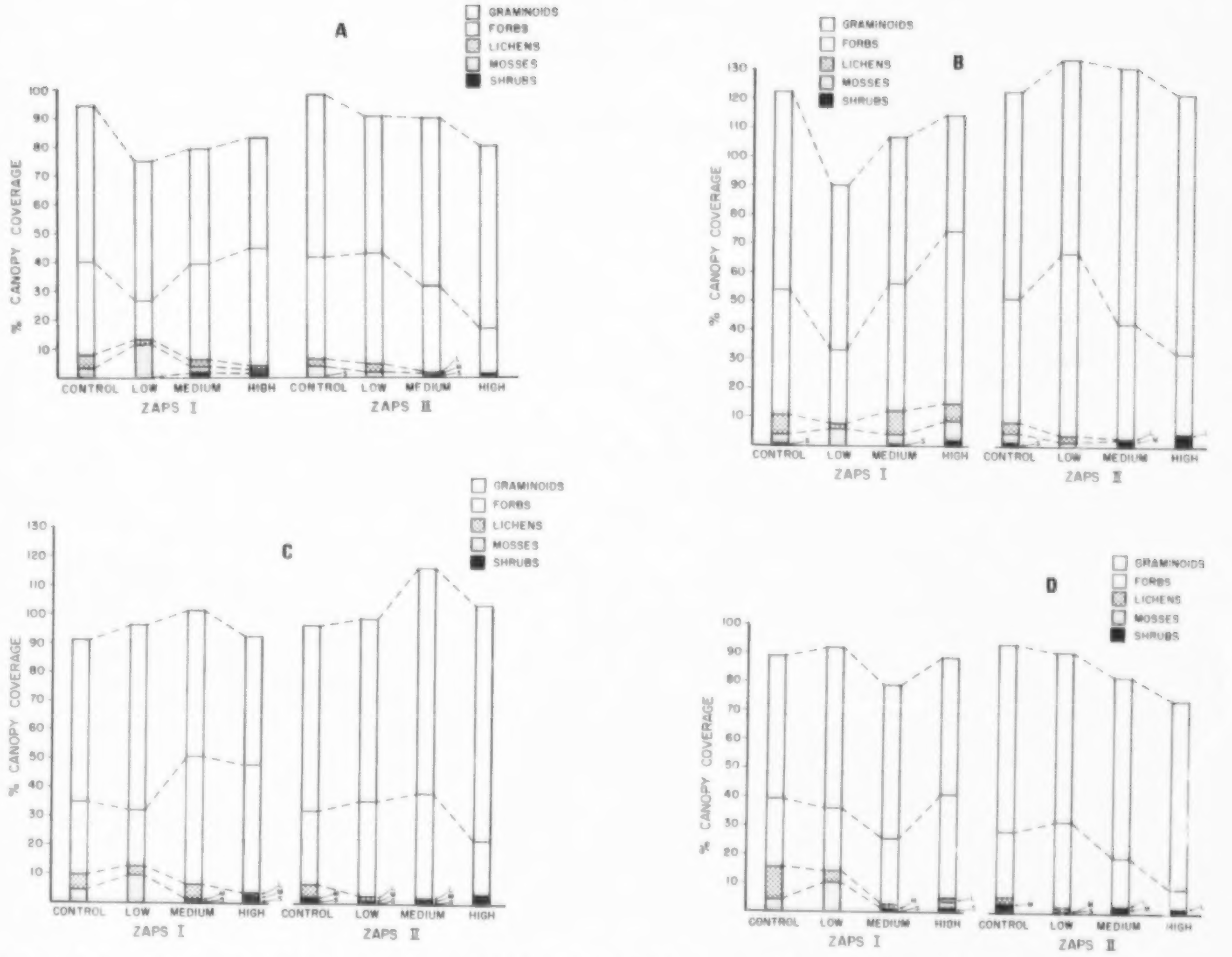


Figure 16.1. Percent canopy coverage, ZAPS I & II, 1977. A=May 21-22; B=June 26-27; C=July 26-27; and D=Sept. 4-5.

There are no strong response patterns in individual grass species on ZAPS I. *Stipa viridula* may be decreasing, but is present in such small amount that trends are difficult to see. The increase in forbs is primarily due to *Taraxacum officinale*. Other species, notably *Achillea millefolium*, appear to respond differently to different treatment rates. This suggests a gradient of interspecific competition across the plots as species are alternatively less and more tolerant than other species to different levels of SO_2 fumigation.

On ZAPS II, the species which are decreasing most strongly are *Agropyron smithii* and *Poa sandbergii*. On the other hand, *Koeleria cristata* is increasing, as is *Stipa viridula*. The latter species is probably responding more to the higher amount of run-in moisture on the high SO_2 plot than to any negative influence of the fumigation. Many of the forbs are present in such low amounts that their treatment responses are obscure. Two species which do show SO_2 effects are *Achillea millefolium* and *Taraxacum officinale*. Both decrease with treatment, although *T. officinale* appears to be slightly enhanced at very low levels of fumigation.

Additional evidence of the fundamental differences between ZAPS I and II is given in Figure 16.2., which shows the numbers of species observed in each sample. On each ZAPS site there are similar seasonal trends in plant numbers. ZAPS I tends to show a depression in species richness at intermediate treatment levels, while ZAPS II responds inversely.

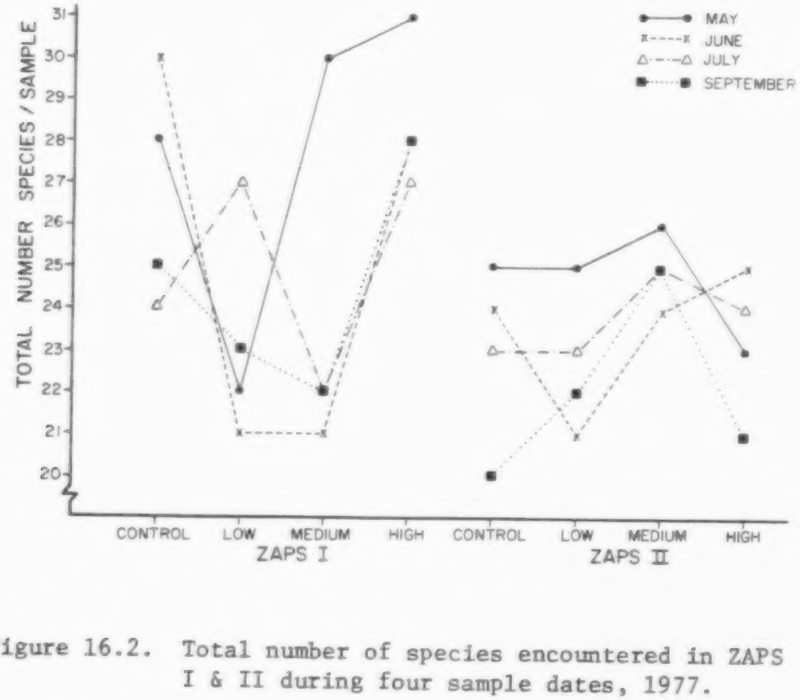


Figure 16.2. Total number of species encountered in ZAPS I & II during four sample dates, 1977.

Diversity

Diversity, evenness, and species richness based on canopy cover are shown in Figure 16.3. The close agreement in diversity trends among dates indicates that diversity can indicate changes in plant community structure. ZAPS I is more diverse than ZAPS II. Both areas show a dip in diversity at the low SO_2 rate. The two components of diversity, evenness and species richness, seem to be about equally influential on both ZAPS areas. Species richness is more variable with season, which is reflected in diversity trends. Evenness is very uniform through seasons, especially on ZAPS II. This suggests that our sampling procedures are very precise, and that interplot differences are real, not sampling artifacts.

When based on plant numbers, very similar trends are seen in diversity and species richness (Figure 16.4., A and C). Evenness is much more variable, which is to be expected because of the high numbers of annuals and *Agropyron smithii* in some of the plots (Figure 16.4., B). The greater overall variability in number-based data is due to the disproportionate influence of species which may be present in high numbers but low coverage or biomass.

Statistical summaries of diversity based on canopy and number are presented in Tables 16.3. and 16.4., respectively. These tables illustrate the greater variation among treatments on ZAPS II and the similar statistical sensitivities of cover- and number-based diversity data.

Table 16.5. compares diversity means based on cover and numbers. The low diversity on the low fumigation level is significant on both ZAPS, but more so on ZAPS II.

A very highly significant correlation exists between diversity based on cover and on number (Figure 16.5.). Further testing may show that either is sufficient to characterize diversity changes due to pollution stress. If only one kind of data were collected, canopy probably would be preferred because it is more closely related to ecological importance (Daubenmire, 1959), is easier to obtain, and is less influenced by periodic flushes of annuals.

We looked at ecosystem stability by comparing standard errors as percents of mean diversity. Values were averaged over all observations within treatments and ZAPS sites. The results are shown in Figure 16.6. There is a tendency for increasing variation with increased stress application on ZAPS II. This may reflect incipient ecosystem instability.

We are convinced that diversity values are showing plant community changes due to air pollution stress. Additional refinements in data collection and analysis will be made in the coming field season. The results will be incorporated in the Power Plant Siting Protocol.

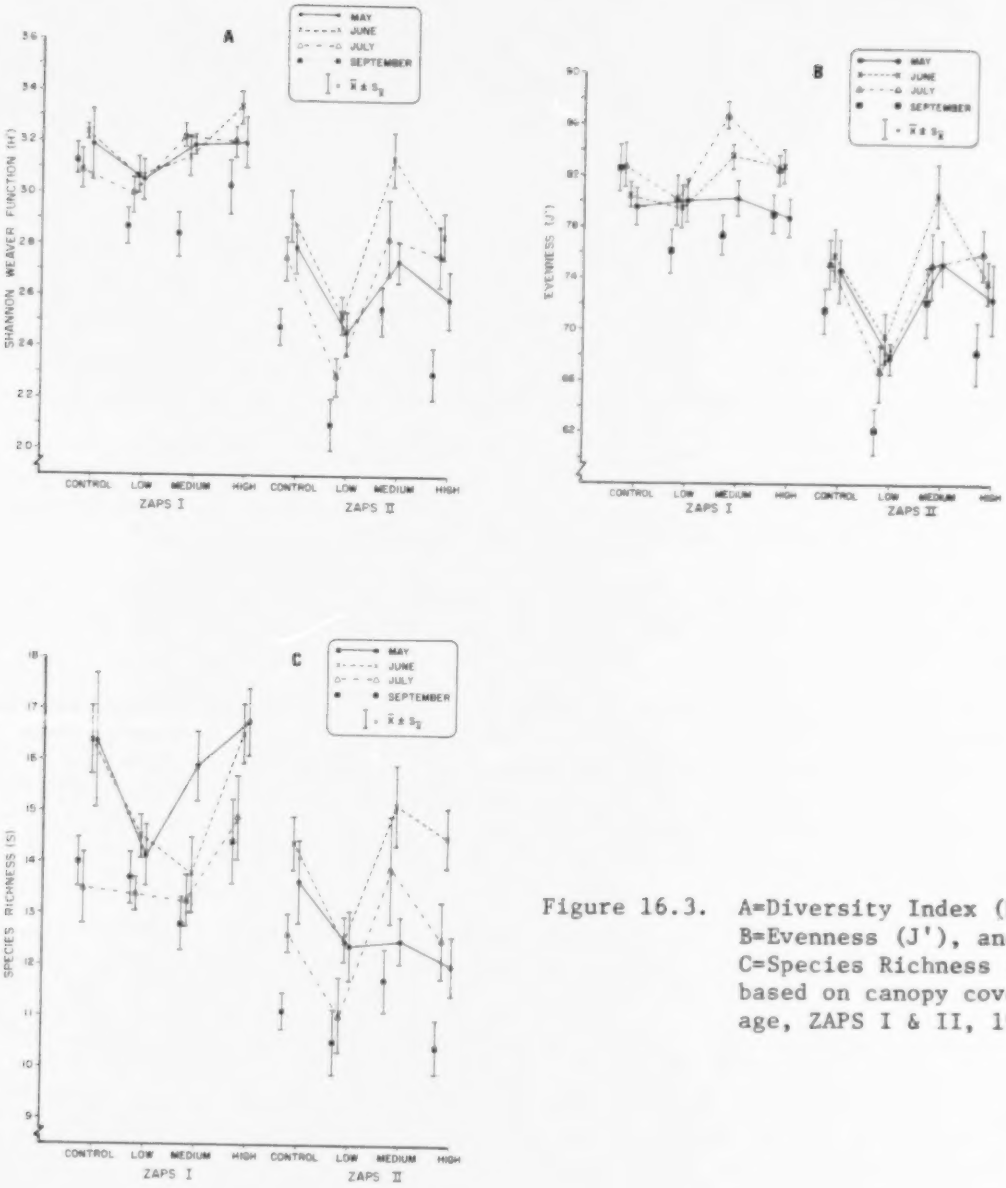
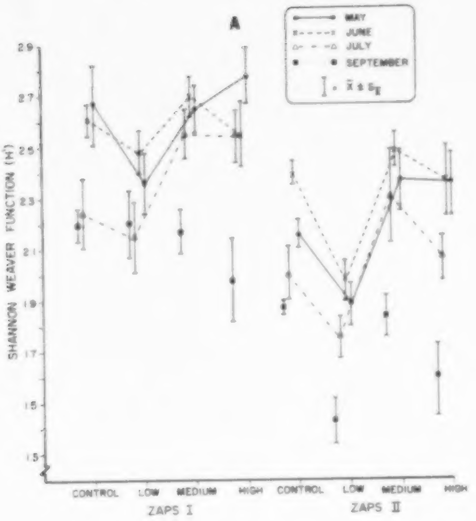
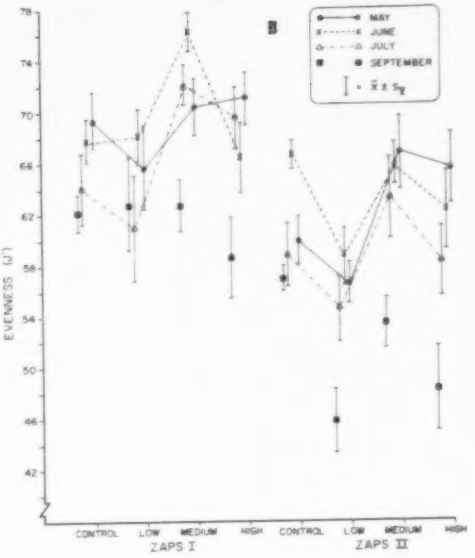


Figure 16.3. A=Diversity Index (H'), B=Evenness (J'), and C=Species Richness (S) based on canopy coverage, ZAPS I & II, 1977.





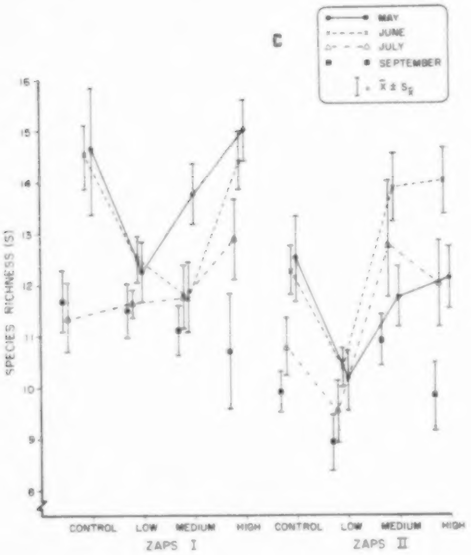


Figure 16.4. A=Diversity Index (H'), B=Evenness (J'), and C=Species Richness (S) based on numbers, ZAPS I & II, 1977.

TABLE 16.3. ANALYSIS OF VARIANCE OF CANOPY COVER BASED DIVERSITY, ZAPS I&II, 1977.

ZAPS I

DATE	SOURCE OF VARIATION	DEGREES FREEDOM	MEAN SQUARE	F
21 MAY	REPLICATIONS	1	1.0593	35.5**
	TREATMENTS	3	.0396	1.3
	GROUPS	3	.0241	.8
	REP X TRMT	3	.1451	4.9*
	REP X GROUP	3	.0172	.6
	TRMT X GROUP	9	.0123	.4
	ERROR	9	.0299	
	TOTAL	31	.0683	
26 JUNE	REPLICATIONS	1	.0000	0.0
	TREATMENTS	3	.1110	3.8*
	GROUPS	3	.0383	1.3
	REP X TRMT	3	.1259	4.4*
	REP X GROUP	3	.0235	.8
	TRMT X GROUP	9	.0112	.4
	ERROR	9	.0289	
	TOTAL	31	.0405	
26-27 JULY	REPLICATIONS	1	.0955	3.3
	TREATMENTS	3	.0921	3.1
	GROUPS	3	.0571	1.9
	REP X TRMT	3	.0402	1.4
	REP X GROUP	3	.0296	1.0
	TRMT X GROUP	9	.0226	.8
	ERROR	9	.0293	
	TOTAL	31	.0393	
4 SEPT	REPLICATIONS	1	.2699	7.6*
	TREATMENTS	3	.1872	5.3*
	GROUPS	4	.0974	2.7
	REP X TRMT	3	.1348	3.8*
	REP X GROUP	4	.1027	2.9
	TRMT X GROUP	12	.0575	1.6
	ERROR	12	.0354	
	TOTAL	39	.0808	

ZAPS II

DATE	SOURCE OF VARIATION	DEGREES FREEDOM	MEAN SQUARE	F
22 MAY	REPLICATIONS	1	.1272	1.5
	TREATMENTS	3	.1854	2.1
	GROUPS	3	.0303	.3
	REP X TRMT	3	.0442	.5
	REP X GROUP	3	.0278	.3
	TRMT X GROUP	9	.0851	1.0
	ERROR	9	.0877	
	TOTAL	31	.0821	
27 JUNE	REPLICATIONS	1	.5422	6.3*
	TREATMENTS	3	.5184	6.0*
	GROUPS	3	.0934	1.1
	REP X TRMT	3	.0218	.3
	REP X GROUP	3	.0388	.5
	TRMT X GROUP	9	.0234	.3
	ERROR	9	.0857	
	TOTAL	31	.1142	
27-28 JULY	REPLICATIONS	1	.4291	4.2
	TREATMENTS	3	.5001	4.9*
	GROUPS	3	.0373	.4
	REP X TRMT	3	.2348	2.3
	REP X GROUP	3	.0385	.4
	TRMT X GROUP	9	.0551	.5
	ERROR	9	.1029	
	TOTAL	31	.1382	
5 SEPT	REPLICATIONS	1	.7020	24.3**
	TREATMENTS	3	.3955	13.7**
	GROUPS	4	.2032	7.0**
	REP X TRMT	3	.0603	2.1
	REP X GROUP	4	.0566	2.0
	TRMT X GROUP	12	.0699	2.4
	ERROR	12	.0288	
	TOTAL	39	.1101	

* Significant at P.05
** Significant at P.01

TABLE 16.4. ANALYSIS OF VARIANCE OF NUMBERS BASED DIVERSITY, ZAPS I & II, 1977.

	ZAPS I					ZAPS II			
DATE	SOURCE OF VARIATION	DEGREES FREEDOM	MEAN SQUARE	F	DATE	SOURCE OF VARIATION	DEGREES FREEDOM	MEAN SQUARE	F
21 MAY	REPLICATIONS	1	.5102	11.5**	22 MAY	REPLICATIONS	1	.6457	6.7*
	TREATMENTS	3	.2487	5.6*		TREATMENTS	3	.4138	4.3*
	GROUPS	3	.0619	1.4		GROUPS	3	.0343	.4
	REP X TRMT	3	.4701	10.6**		REP X TRMT	3	.0433	.5
	REP X GROUP	3	.1456	3.3		REP X GROUP	3	.1063	1.1
	TRMT X GROUP	9	.0345	.8		TRMT X GROUP	9	.0192	.2
	ERROR	9	.0445			ERROR	9	.0962	
	TOTAL	31	.1290			TOTAL	31	.1122	
26 JUNE	REPLICATIONS	1	.0341	.4	27 JUNE	REPLICATIONS	1	.7532	47.2**
	TREATMENTS	3	.0671	.8		TREATMENTS	3	.4132	25.9**
	GROUPS	3	.1010	1.1		GROUPS	3	.0346	2.2
	REP X TRMT	3	.1076	1.2		REP X TRMT	3	.1052	6.6*
	REP X GROUP	3	.0376	.4		REP X GROUP	3	.0825	5.2*
	TRMT X GROUP	9	.0420	.5		TRMT X GROUP	9	.0197	1.2
	ERROR	9	.0879			ERROR	9	.0160	
	TOTAL	31	.0691			TOTAL	31	.0961	
26-27 JULY	REPLICATIONS	1	.3116	2.7	27-28 JULY	REPLICATIONS	1	.8511	10.6**
	TREATMENTS	3	.3409	2.9		TREATMENTS	3	.4145	5.2*
	GROUPS	3	.1353	1.2		GROUPS	3	.0354	.4
	REP X TRMT	3	.0996	.9		REP X TRMT	3	.3807	4.7*
	REP X GROUP	3	.1605	1.4		REP X GROUP	3	.0352	.4
	TRMT X GROUP	9	.0682	.6		TRMT X GROUP	9	.0458	.6
	ERROR	9	.1172			ERROR	9	.0804	
	TOTAL	31	.1351			TOTAL	31	.1479	
4 SEPT	REPLICATIONS	1	.1865	1.9	5 SEPT	REPLICATIONS	1	.5068	20.1**
	TREATMENTS	3	.1102	1.1		TREATMENTS	3	.4342	17.2**
	GROUPS	4	.1653	1.7		GROUPS	4	.0271	1.1
	REP X TRMT	3	.4603	4.7*		REP X TRMT	3	.1298	5.1*
	REP X GROUP	4	.0252	.3		REP X GROUP	4	.0321	1.3
	TRMT X GROUP	12	.1394	1.4		TRMT X GROUP	12	.1349	5.3**
	ERROR	12	.0974			ERROR	12	.0253	
	TOTAL	39	.1411			TOTAL	39	.1117	

* Significant at P.05
** Significant at P.01

TABLE 16.5. MEAN VALUES OF DIVERSITY (H') BASED ON CANOPY COVERAGE AND NUMBERS, ZAPS I & II, 1977.

ZAPS I				ZAPS II			
DATE	TREATMENT	CANOPY MEAN	NUMBERS MEAN	DATE	TREATMENT	CANOPY MEAN	NUMBERS MEAN
21 MAY	CONTROL	3.1899	2.6659 a[1]	22 MAY	CONTROL	2.7921	2.1617 ab
	LOW	3.0514	2.3632 b		LOW	2.4542	1.8835 a
	MEDIUM	3.1898	2.6525 a		MEDIUM	2.7365	2.3691 b
	HIGH	3.1962	2.7757 a		HIGH	2.5894	2.3585 b
26 JUNE	CONTROL	3.2367 ab	2.6044	27 JUNE	CONTROL	2.9137 a	2.4003 a
	LOW	3.0659 a	2.4786		LOW	2.5217 b	1.9777 b
	MEDIUM	3.1435 ab	2.6968		MEDIUM	3.1373 a	2.4910 a
	HIGH	3.3385 b	2.5536		HIGH	2.8419 ab	2.3704 a
26-27 JULY	CONTROL	3.0929	2.2427	27-28 JULY	CONTROL	2.7459 a	2.0095 ab
	LOW	2.9924	2.1527		LOW	2.2854 b	1.7558 a
	MEDIUM	3.2249	2.5528		MEDIUM	2.8334 a	2.3104 b
	HIGH	3.2023	2.5463		HIGH	2.7593 a	2.0663 ab
4 SEPT	CONTROL	3.1324 a	2.1961	5 SEPT	CONTROL	2.4781 a	1.8741 a
	LOW	2.8681 b	2.2044		LOW	2.1031 b	1.4341 b
	MEDIUM	2.8429 b	2.1733		MEDIUM	2.5468 a	1.8352 a
	HIGH	3.0270 ab	1.9830		HIGH	2.2980 c	1.5908 c

---

[1] Any two means within dates and columns not followed by the same letter are significantly different at the .05 level.

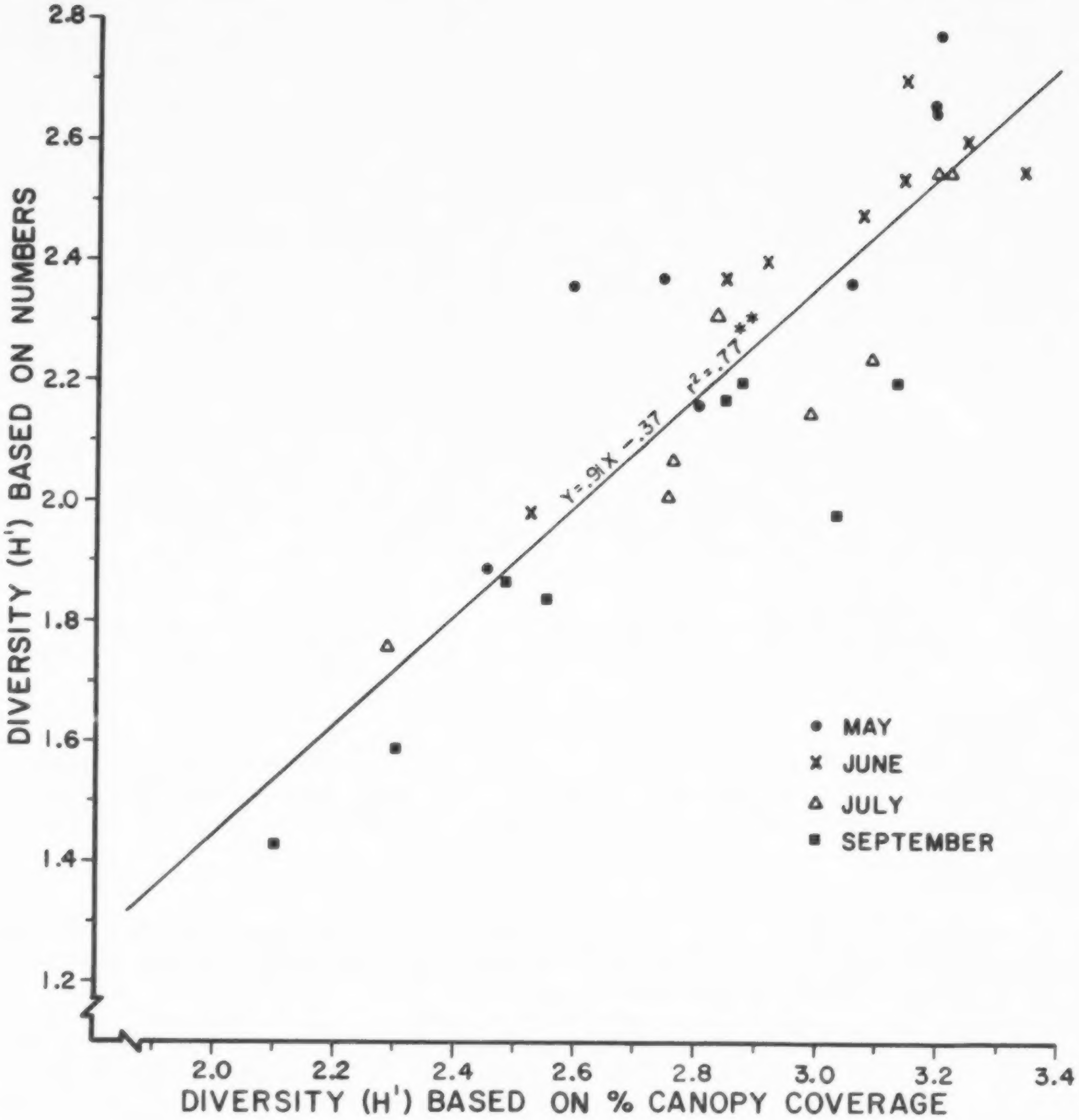


Figure 16.5. The regression of diversity based on numbers onto diversity based on canopy coverage, ZAPS I and II, 1977.
** $P < .01$.

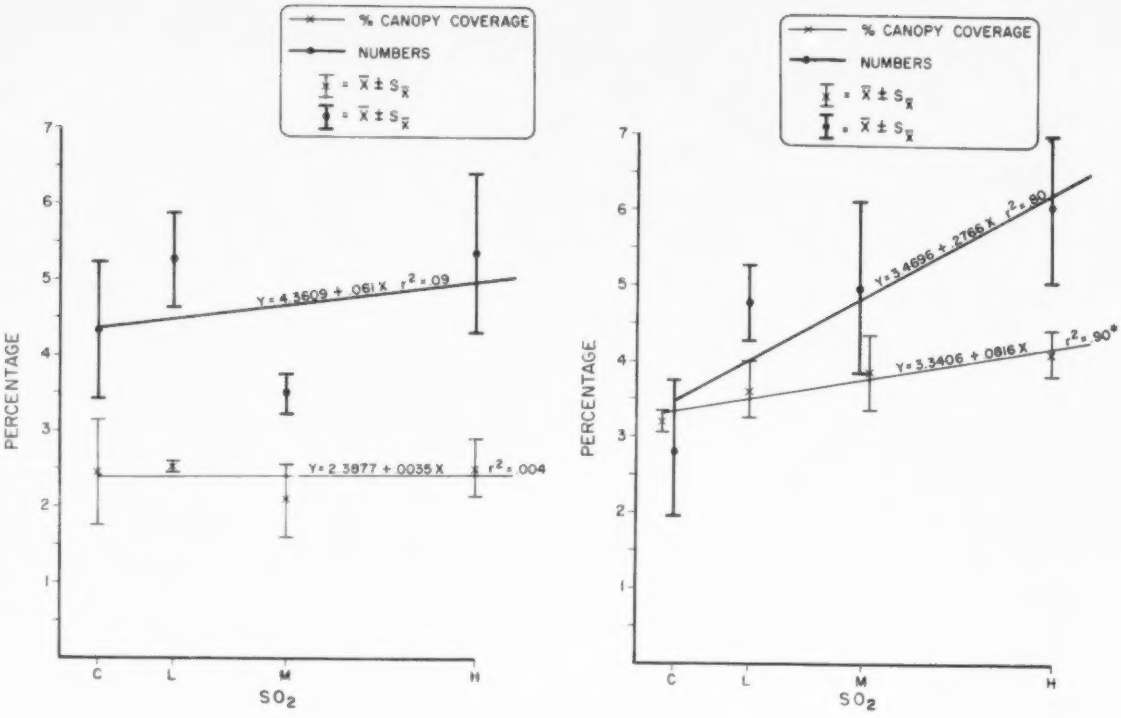


Figure 16.6. Standard errors expressed as percentages of mean diversity over four observations, ZAPS I (left) and ZAPS II (right), 1977.

Phenology

Phenological data from the ZAPS sites are presented in Appendices 16.2 and 16.3. No conspicuous trends are shown. These data are contributions to the long-term phenologic baseline records of the sites.

Lichens

Although lichens usually are ignored in grassland research, they may be important on pollution studies because they are more sensitive than other plant groups. (LeBlanc and Rao, 1975).

We have traced lichen canopy cover since our first data collections on ZAPS I in 1975, and ZAPS II in 1976 (Figures 16.7. and 16.8.). The graphs are based on averages among sample dates in each year. The principal lichen is *Parmelia chlorochroa* on both ZAPS sites. Our earlier data may include small amounts of blue-green algae, but not in sufficient quantities to influence conclusions.

In 1975, the first year of stress on ZAPS I, lichens were not depressed by SO_2 fumigation. The low treatment plot had a significantly lower lichen cover than the other three plots, probably because of inherent site differences. In the second year, a significant depression in lichens was

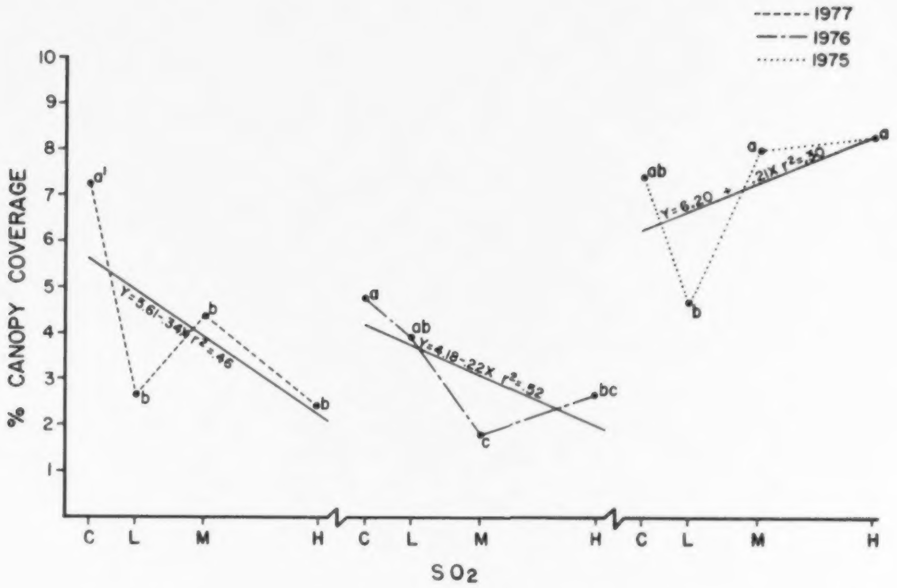


Figure 16.7. Mean lichen coverage (canopy) for ZAPS I, 1977, 76, and 75. [1]Means within years followed by the same letter are not significantly different at the p.05 level.

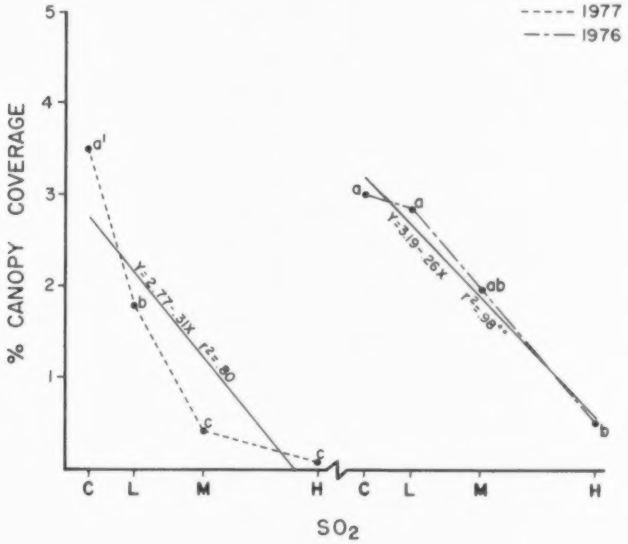


Figure 16.8. Mean lichen coverage (canopy) for ZAPS II, 1977 and 1976. [1]Means within years followed by the same letter are not significantly different at the p.05 level.
** P < .01

observed with increasing stress. The overall reduction in cover is ascribed to the dry growing conditions in 1976. By 1977, which was a more normal moisture year, the cover of lichens was significantly lower on all levels of SO_2 compared with the control. The lower inherent lichen cover on the low treatment plot greatly decreases the linearity of the regression.

The situation on ZAPS II is different. This may be due to a later first sample date, more intense and earlier fumigation, and the less favorable growing conditions in 1976. The highly significant linear regression shows the depression of lichen cover in the first year of fumigation. In 1977, the second year of treatment stress, lichen cover was significantly depressed at all SO_2 levels. The data points would be better fit by an exponential curve, which is typical of biological functions.

In an attempt to eliminate annual climatic effects, we normalized the data by expressing treatment coverages as percents of controls (Figure 16.9.). The increasing depression in lichen cover with years is clearly illustrated, as is the consistently low lichen cover on the 2pphm SO_2 treatment.

The ZAPS II curves reflect the more severe lichen losses in both years. They also more clearly show the overall loss the second year.

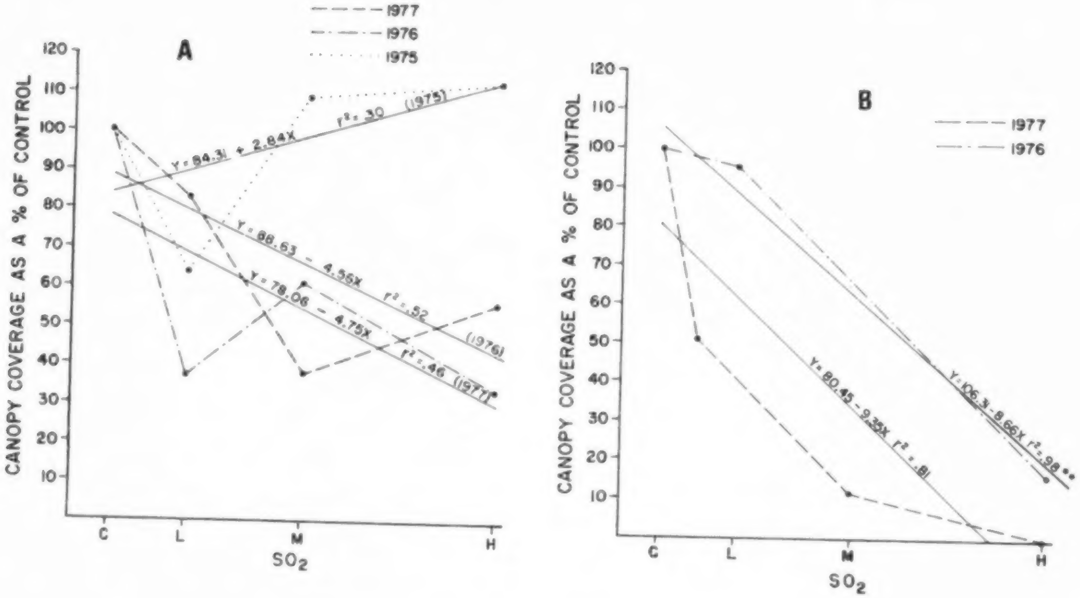


Figure 16.9. Lichen cover in four treatments expressed as a percentage of the control (0 pphm SO_2), ZAPS I (A) and ZAPS II (B).
** $P < .01$

A better way to elucidate temporal changes is to express lichen cover as a percentage of first year cover for each treatment (Figure 16.10.). Both ZAPS I and II have lost very substantial portions of their original lichen cover, and this is related to SO_2 level. The great negative slope on ZAPS II illustrates the more severe nature of the stress being applied there. Even the low (2pphm) exposure level has reduced lichens to about 60% of the original cover on both sites.

Summaries of analysis of variance for lichen data are given in Table 16.6. In every case, treatment effects become more significant with additional seasons of stress.

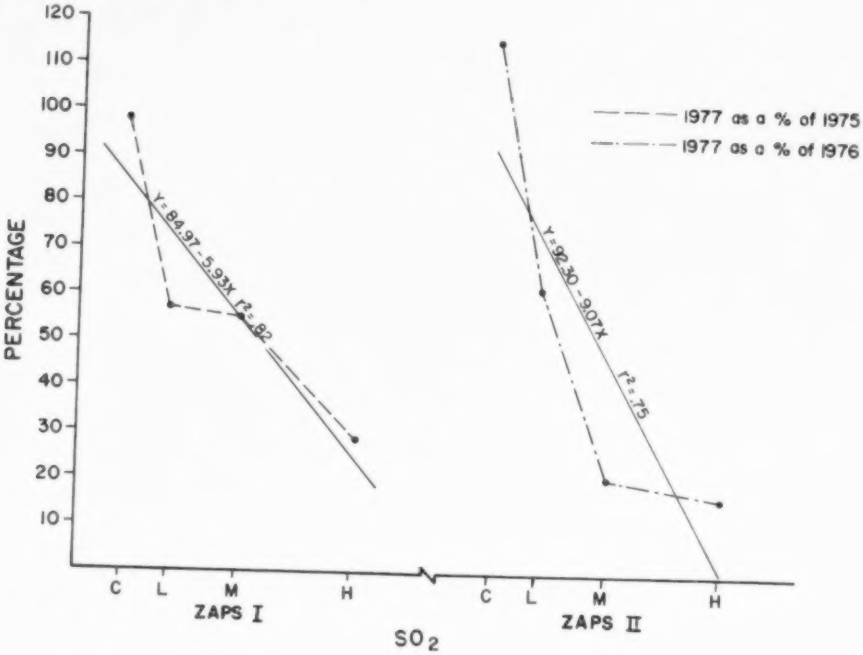


Figure 16.10. Lichen cover expressed as a percentage of the first year coverage.

CONCLUSIONS

Species composition, based on both canopy cover and number, varies among SO_2 rates and sites. On ZAPS I graminoids are being replaced by forbs at higher fumigation levels. The opposite response is occurring on ZAPS II. The relative importance of treatments and inherent site variability is still unclear.

Species diversity is sensitive in monitoring seasonal plant community changes. Low standard errors and large differences in sample means suggests that the treatment plots are different. Evenness and species richness is much more variable among sample dates.

TABLE 16.6. ANALYSIS OF VARIANCE FOR LICHEN COVER, ZAPS I & II.

ZAPS I					ZAPS II				
YEAR	SOURCE VARIATION	DEGREES FREEDOM	MEAN SQUARE	F	YEAR	SOURCE OF VARIATION	DEGREES FREEDOM	MEAN SQUARE	F
1977	REPLICATIONS	1	128.1177	5.8*	1977	REPLICATIONS	1	.0000	.0
	TREATMENTS	3	177.3382	8.0**		TREATMENTS	3	77.1520	42.9**
	GROUPS	4	58.5180	2.7*		GROUPS	4	.3630	.2
	OBSERVATIONS	3	61.6302	2.8*		OBSERVATIONS	3	2.0755	1.2
	TRMT X OBSERV	9	26.8055	1.2		TRMT X OBSERV	9	2.6797	1.5
	TRMT X REP	3	14.3382	.7		TRMT X REP	3	2.9167	1.6
	TRMT X GROUPS	12	35.7682	1.6		TRMT X GROUPS	12	.6285	.4
	OBSERV X REP	3	42.0678	1.9		OBSERV X REP	3	5.9192	3.3*
	OBSERV X GROUPS	9	8.8924	.4		OBSERV X GROUPS	9	1.6050	.9
	REP X GROUPS	4	40.3179	1.8		REP X GROUPS	4	1.0191	.6
	ERROR	84	22.0610			ERROR	84	1.7968	
	TOTAL	135	31.7832			TOTAL	135	3.4847	
1976	REPLICATIONS	1	1.0000	.2	1976	REPLICATIONS	1	3.0625	.6
	TREATMENTS	3	27.1823	4.1*		TREATMENTS	3	21.0052	4.2*
	GROUPS	3	4.5677	.7		GROUPS	3	1.6406	.3
	OBSERVATIONS	1	31.6406	4.7*		OBSERVATIONS	1	66.0156	13.1**
	TRMT X OBSERV	3	23.2240	3.5*		TRMT X OBSERV	3	3.5469	.7
	TRMT X REP	3	11.6042	1.7		TRMT X REP	3	9.4271	1.9
	TRMT X GROUPS	9	2.6788	.4		TRMT X GROUPS	9	3.6719	.7
	OBSERV X REP	1	16.0000	2.4		OBSERV X REP	1	1.5625	.3
	OBSERV X GROUPS	3	9.6719	1.4		OBSERV X GROUPS	3	3.5156	.7
	REP X GROUPS	3	14.0729	2.1		REP X GROUPS	3	6.3750	1.3
	ERROR	33	6.6746			ERROR	33	5.0420	
	TOTAL	63	8.9521			TOTAL	63	6.4540	
1975	REPLICATIONS	1	7.7521	.3					
	TREATMENTS	3	81.7354	3.1*					
	GROUPS	4	1.3042	.1					
	OBSERVATIONS	2	49.3188	1.9					
	TRMT X OBSERV	6	99.6687	3.8**					
	TRMT X REP	3	25.9799	1.0					
	TRMT X GROUPS	12	19.1694	.7					
	OBSERV X REP	2	25.9522	1.0					
	OBSERV X GROUPS	8	22.8994	.9					
	REP X GROUPS	4	27.0854	1.0					
	ERROR	74	26.0416						
	TOTAL	119	29.6917						

* Significant at P.05.
** Significant at P.01.

Differences in phenology among treatment plots were not seen, either because phenology is not affected or because of the masking influence of climate, sites, and grazing history. Long-term baseline records are necessary for phenologic comparisons.

Lichen cover is decreasing with increasing SO_2 rates, and this effect is cumulative with years.

REFERENCES

Daubenmire, R.F. 1959. A Canopy-Covered Method of Vegetational Analysis. Northw. Sci., 33(1):43-64.

Eversman, S. 1978. Effects of Low-Level SO_2 Stress on Two Lichen Species. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Third Interim Report, Colstrip, Montana. E.M. Preston and R.A. Lewis, eds. EPA-600/3-78-021, U.S. Environmental Protection Agency, Corvallis, Oregon, pp. 385-398.

LeBlanc, F. and D.N. Rao, 1975. Effects of Air Pollutants on Lichens and Bryophytes. In Mudd, J.B. and T.T. Kozlowski (Eds). Responses of Plants to Air Pollution. Academic Press, New York. pp. 237-272.

APPENDIX 16.1.

SPECIES ENCOUNTERED ON THE TWO ZAP SITES

SYMBOL	SPECIES	ZAPS I				ZAPS II			
		Cont.	Low	Med.	High	Cont.	Low	Med.	High
	GRAMINOIDS								
AGSM	*Agropyron smithii*	X	X	X	X	X	X	X	X
AGSP	*A. spicatum*			X					X
ARLO	*Aristida longiseta*	X	X	X	X	X		X	
BOGR	*Bouteloua gracilis*	X	X	X	X	X	X	X	X
BRJA	*Bromus japonicus*	X	X	X	X	X	X	X	X
BRTE	*B. tectorum*	X	X	X	X	X	X	X	X
BUDA	*Buchloe dactyloides*				X				
CAMO	*Calamagrostis montanensis*	X	X	X	X			X	
CAFI	*Carex filifolia*			X				X	X
CAPE	*C. pennsylvanica*		X	X	X			X	X
DAUN	*Danthonia unispicata*	X			X				
FEID	*Festuca idahoensis*	X	X	X	X				
JUIN	*Juncus interior*	X	X	X	X				
KOCR	*Koeleria cristata*	X	X	X	X	X	X	X	X
MUCU	*Muhlenbergia cuspidata*			X				X	
PHPR	*Phleum pratense*		X						
POPR	*Poa pratensis*	X	X	X	X	X	X	X	X
POSA	*P. sandbergii*	X	X	X	X	X	X	X	X
SCPA	*Schedonnardus paniculatus*			X	X	X	X	X	X
SPCR	*Sporobolus cryptandrus*	X	X	X	X		X		X
STCO	*Stipa comata*	X	X	X	X	X	X	X	X
STVI	*S. viridula*	X	X	X	X	X	X	X	X
VUOC	*Vulpia octoflora*	X	X	X	X	X	X		
	FORBS								
ACMI	*Achillea millefolium*	X	X	X	X	X	X	X	X
AGGL	*Agoseris glauca*	X			X				
ALTE	*Allium textile*	X	X	X		X	X	X	X
AMPS	*Ambrosia psilostachya*	X	X	X	X				X
ANOC	*Androsace occidentalis*	X	X	X	X	X	X	X	X
ANTEN	*Antennaria* species	X	X	X	X	X	X	X	
ANNE	*A. neglecta*	X	X	X	X				
ANRO	*A. rosea*	X	X	X	X		X		X
ARHO	*Arabis holboellii*	X		X					
ARSO	*Arnica sororia*	X	X	X	X	X	X		
ARLU	*Artemisia ludoviciana*	X	X	X	X	X		X	X
ASPUM	*Asclepias pumila*			X					
ASTER	*Aster* species			X		X	X	X	X

APPENDIX 16.1. (continued)

SPECIES ENCOUNTERED ON THE TWO ZAP SITES

		ZAPS I				ZAPS II			
SYMBOL	SPECIES	Cont.	Low	Med.	High	Cont.	Low	Med.	High
ASFA	*A. falcatus*		X				X	X	
ASTRA	*Astragalus* species		X	X	X			X	
ASCR	*A. crassicarpus*	X	X	X	X			X	
ASDA	*A. drummondii*				X	X		X	
ASGI	*A. gilviflorus*			X					
ASPU	*A. purshii*	X	X	X		X		X	
BAOP	*Bahia oppositifolia*	X				X	X	X	X
BEWY	*Besseya wyomingensis*	X		X	X	X	X	X	X
CAMI	*Camelina microcarpa*					X		X	
CANU	*Calochortus nuttallii*			X					
CEAR	*Cerastium arvense*	X	X	X	X	X	X	X	
CHAL	*Chenopodium album*		X	X			X	X	X
CHTE	*Chorispora tenella*					X	X		X
CIUN	*Cirsium undulatum*		X	X	X				
COLI	*Collomia linearis*	X							
COCA	*Conyza canadensis*	X	X	X	X	X	X	X	
CREPI	*Crepis* species		X					X	
DEBI	*Delphinium bicolor*	X	X	X	X				
DESCU	*Descurainia* species							X	
DRABA	*Draba* species	X	X	X	X				
ECPA	*Echinacea pallida*				X				
ERDI	*Erigeron divergens*	X	X	X	X	X	X	X	X
ERPU	*E. pumilus*		X	X	X				
ERAS	*Erysimum asperum*	X	X	X	X				
GACO	*Gaura coccinea*	X	X	X	X	X		X	
GETR	*Geum triflorum*				X				
GRSQ	*Grindelia squarrosa*	X	X	X	X	X	X	X	X
HASP	*Haplopappus spinulosus*			X					
HEHI	*Hedeoma hispida*	X	X	X	X	X	X	X	X
HEVI	*Heterotheca villosa*	X	X	X	X		X	X	
LASE	*Lactuca serriola*				X	X	X	X	X
LAEC	*Lappula enchinata*					X		X	
LEDE	*Lepidium densiflorum*	X		X	X	X	X	X	X
LEMO	*Leucocrinum montanum*	X	X	X	X			X	X
LIIN	*Lithospermum incisum*				X				
LIRU	*L. ruderale*					X			
LOMAT	*Lomatium* species	X		X	X	X	X	X	X
LOOR	*L. orientale*	X			X				X
LUPIN	*Lupinus* species		X	X					
LUSE	*L. sericeus*			X					
MAMI	*Mammillaria missouriensis*	X	X	X	X	X	X	X	

APPENDIX 16.1. (continued)

SPECIES ENCOUNTERED ON THE TWO ZAP SITES

		ZAPS I				ZAPS II			
SYMBOL	SPECIES	Cont.	Low	Med.	High	Cont.	Low	Med.	High
MESA	*Medicago sativa*				X				
MEAL	*Melilotus alba*				X				
MEOF	*M. officinalis*	X	X	X	X	X	X	X	X
MERTE	*Mertensia* species				X				
MEOB	*M. oblongifolia*							X	
MOFI	*Monarda fistulosa*	X	X		X				
OPFR	*Opuntia fragilis*	X	X	X	X	X	X	X	X
OPPO	*O. polyacantha*	X	X		X	X	X	X	X
ORLU	*Orthocarpus luteus*	X	X	X	X	X	X	X	X
OXSE	*Oxytropis sericea*			X					
PENST	*Penstoman* species		X						
PENI	*P. nitidus*		X			X			
PEPU	*Petalostemon purpureum*		X	X					
PHHO	*Phlox hoodii*	X	X	X	X	X	X	X	X
PLPA	*Plantago patagonica*	X	X	X	X	X	X	X	X
PLSP	*P. spinulosa*	X	X	X	X	X	X	X	X
POVI	*Polygonum viviparum*	X	X	X	X		X		
PSAR	*Psoralea argophylla*	X	X	X	X			X	
PSES	*P. esculenta*					X		X	
RAGL	*Ranunculus glaberrimus*	X			X		X		
RACO	*Ratibida columnifera*	X	X	X	X		X	X	X
SENEC	*Senecio* species	X		X					
SIAN	*Sisyrinchium angustifolium*				X				
SOLID	*Solidago* species			X	X			X	
SOMI	*S. missouriensis*		X	X	X			X	X
SPCO	*Sphaeralcea coccinea*	X	X	X	X	X	X	X	X
TAOF	*Taraxacum officinale*	X	X	X	X	X	X	X	X
THAR	*Thlaspi arvense*		X						
TRDU	*Tragopogon dubius*	X	X	X	X	X	X	X	X
VIAM	*Vicia americana*	X		X	X	X	X	X	X
VINU	*Viola nuttallii*						X		X
ZYVE	*Zygadenus venenosus*	X	X	X	X	X		X	X
	HALF-SHRUBS AND SHRUBS								
ARCA	*Artemisia cana*	X	X	X	X		X	X	X
ARDR	*A. dracunculus*		X						
ARFR	*A. frigida*	X	X	X	X	X	X	X	X
ARTR	*A. tridentata*	X	X	X	X	X	X	X	X
ATNU	*Atriplex nuttallii*				X	X			X
CELA	*Ceratoides lanata*	X				X		X	X
XASA	*Xanthocephalum sarothrae*	X	X	X	X	X		X	X

APPENDIX 16.2.
PHENOLOGICAL PROFILE FOR ZAPS I, 1977[1]

SPECIES	CONTROL						LOW						MEDIUM						HIGH					
	4-13	4-26	6-2	6-26	7-19	9-4	4-13	4-26	6-2	6-26	7-19	9-4	4-13	4-26	6-2	6-26	7-19	9-4	4-13	4-26	6-2	6-26	7-19	9-4
GRAMINOIDS																								
Agropyron smithii	1	2	3-4	7	4	10	1	2	3	4	8	10	1	3	3	3 some 8	8	9	2	2	3	4&7	4&9	4&10
A. spicatum														3	6	9	11	12						
Aristida longiseta	12	1	3	6	11	12	12	1	3	7	9	12	12	1	3	6	11	11	12	1	3	6	11	12
Bouteloua gracilis	12			7	8	12	12	1	3	7	9	12	12	12		7		11	12	12	3	7	8	11
Bromus japonicus	1	2	7	9	11	11-12	2	3	7	10	11	11-12	1	2	6	10	11	12		2	6	9	11	12
B. tectorum	1	2			11		2	3		11		11-12									6	10	11	12
Buchloe dactyloides																					3	9	10	12
Calamagrostis montanensis	2			10		12		2		4	11	12		3		3						4		
Carex filifolia													2	8	11	12	12	12						
C. pennsylvanica							2	3	4	12	12	12		2	4	11	12	3						3
Danthonia unispicata			8	10	12	12&3																11		12
Festuca idahoensis		4	7	9	11	12			7	9	12	12				10		12			7			12
Juncus interior			6	8		12																		12
Koeleria cristata	2	3	7-8	10	11	12	2	3	7-8	10	11	12	2	3	8-9	11	11	12			8-9	11	12&3	12
Muhlenbergia cuspidata																	8	11						
Poa pratensis	3	3	8	10	11	12	3	3	8	10	11	12	3	3	8	9	11	11	3	3	8	10	11	11
P. sandbergii	3	3	8	10	11	12	3	3	8	10	11	12	3	3	8	9	11	12	3	3	9	11	12	12
Sporobolus cryptandrus	12	1			6		12	1	3	3	5					3	5	11						
Stipa comata	1	2	5	11	11	12	1	2	4	10	11	12		2	5	10	11	12				11	11	12
S. viridula		3	5	10	12	12		2			11	12	1	3	6	11	12	3		3	6	10	11	3
Vulpia octoflora						12						12												
FORBS																								
Achillea millefolium	1	3	6	8	9	11-12	1	3	7	8	9	12&3	1	3	9	9	10	3	2	3	9	9	11	12&3
Agoseris glauca																				2				
Allium textile						12																		
Ambrosia psilostachya					8							10					6							10
Androsace occidentalis						12						12												

---

[1] Codes are given on p 110.

APPENDIX 16.2. (continued)

SPECIES	CONTROL						LOW						MEDIUM						HIGH					
	4-13	4-26	6-2	6-26	7-19	9-4	4-13	4-26	6-2	6-26	7-19	9-4	4-13	4-26	6-2	6-26	7-19	9-4	4-13	4-26	6-2	6-26	7-19	9-4
FORBS (continued)																								
Antennaria sp.	1	4	9	12	12	12	1	5	9	12	12		1	4	11	12	12	12	1		10	11-12	12	12
Arabis holboellii						11								12										
Arnica sororia	2	2	8	12	12	12		2	8	12	12	12								2	8	12	12	12
Artemisia ludoviciana	12		3	3	5	8	12	2	3	3	5	8	12	2	3	3	4	4		2		3	4	8
Asclepias pumila														3		4								
Aster falcatus								1	3	4	9	9												
Astragalus sp.																								12
A. crassicarpus								1		9	11	12	1	3	9	9	11	12		3	9	9	11	12
A. drummondii																					9	9		
A. gilviflorus													1			4								
A. purshii					11										9		11	12						
Besseya wyomingensis		6	9											8	9	12	12	12		6	9	12	12	12
Cerastium arvense	1	3	9	12	12	12			9		12	12	2	3	9		12	12			9		12	12
Chenopodium album																	8	12						
Cirsium undulatum								1					12		4	3	3	11&3			3	4		11&3
Collomia linearis			8																					
Conyza canadensis				5		12				5		12											7	
Crepis sp.										11														
Delphinium bicolor																			3	3				
Descurainia sp.		4				12	2	4				12												
Echinacea pallida																					6	8-9	11	12
Erigeron divergens		3	8	9	8-11	12&3	2	3	8	9	9-11	12	2	3	9	9	9&1	10&3	3	2	8	9	7-12	8-12&3
Erysimum asperum			2			12				9					9	10								
Gaura coccinea															5									
Grindelia squarrosa	12	3	3	4	5	9-10			4	5	6	11		2								4	7	9&3
Haplopappus spinulosus																5	6	9						
Hedeoma hispida				7		12				9		12												
Heterotheca villosa								2	4	8	9	11		2			7				4		8	9
Lactuca serriola																				2				12
Lepidium densiflorum						12									9									
Leucocrinum montanum						12		3	10	12		12	2	4	11	12	12	12		4	11	12	12	12
Lithospermum incisum																							10	
Lomatium sp.														9			12	12	4	7	12	12	12	12

APPENDIX 16.2. (continued)

	CONTROL						LOW						MEDIUM						HIGH					
SPECIES	4-13	4-26	6-2	6-26	7-19	9-4	4-13	4-26	6-2	6-26	7-19	9-4	4-13	4-26	6-2	6-26	7-19	9-4	4-13	4-26	6-2	6-26	7-19	9-4
FORBS (continued)																								
L. orientale		8	12																					
Lupinus sp.															6	8	11	12						
L. sericeus																	8	12						
Mammilaria missouriensis						12		1		11	12													
Medicago sativa																						8	9	11
Melilotus officinale		1	6	8	11	12		3	6	8&1	11	11-12	12		6	8	11	11	12	1	6	8&1	11	11&3
Monarda fistulosa						3					3													
Opuntia fragilis		1	4	5	12	12		1			12	12		1	4	4	12	12		1			12	12
O. polyacantha	1	1				12						12												
Orthocarpus luteus	12	12	3	8		12				8	8-11	12	12		4	8	8-11	12			4	8	9	12
Oxytropis sericea																11		12						
Petalostemon purpureum																11								
Phlox hoodii		8			12	12						12	3	8	12	12	12	12						
Plantago patagonica	12	12	6	8	8	12	12		6	8	8	12	12				9	12	12		6	8	9	12
P. spinulosa	12			8	8	12	12					12	12				8	12	12		6	8	9	12
Polygonum viviparum				9	11	12				10		12											10	
Psoralea argophylla			5	9	10	12			5	9	9	11	12		4	9	10	12			6	9	10	12
Ranunculus glaberrimus	6	8																	5	8				
Ratibida columnifera	12			6	8	11	12	1	5	6	9	12	12		5	6	9	11		12	5	6	8	11
Senecio sp.			8												9	10								
Sisyrinchium angustifolium																					8			
Solidago sp.																				12	3	3	6	9
S. missouriensis														12		4	6	8		1	3	5	6	9
Sphaeralcea coccinea		1	5	10	10	12			5	9	11	12		1	5	10	11	12			5	10	11	12
Taraxacum officinale	1	5	12	12	12	12&3	1	5	12	12&1	12&3	12&3	2	5	12	12	12	12&3	2	5	12	12&1	12	12&3
Thlaspi arvense										9														
Tragopogon dubius	1	3	8	11	12&3	12&3	1	3	8	12&3	12&3	12&3	2	3	8	11&3	12	12&3	1			11&3	12	12&3
Vicia americana		3	9	11	12	12									9	11	12	12						
Zygadenus venenosus		3				12	3	4	9	12	12	12	3	4	9	11	12	12		3	9	12	12	12

APPENDIX 16.2. (continued)

SPECIES	CONTROL 4-13	4-26	6-2	6-26	7-19	9-4	LOW 4-13	4-26	6-2	6-26	7-19	9-4	MEDIUM 4-13	4-26	6-2	6-26	7-19	9-4	HIGH 4-13	4-26	6-2	6-26	7-19	9-4
HALF SHRUBS AND SHRUBS																								
Artemisia cana			3	3	4	10		3	3	3	4	10			3		3	9			3	3	5	8
A. dracunculus								1		3														
A. frigida	1	1	3	3	5	10	1	1	3	3	5	10	1	1	3	3	4	8	1	1	3	3	5	9
A. tridentata	1	2	3	3	5	10	1	3	3	3	5	10	1	3	3	3	4	9	1	3	3	3	5	9
Xanthocephalum sarothrae	1	2	3	3	5	10		2				9	1	2	3	3	4	8	1	1		3	4	9

APPENDIX 16.3.
PHENOLOGICAL PROFILE FOR ZAPS II, 1977[1]

SPECIES	CONTROL						LOW						MEDIUM						HIGH					
	4-13	4-26	5-26	6-26	7-26	9-5	4-13	4-26	5-26	6-26	7-28	9-5	4-13	4-26	5-26	6-26	7-28	9-5	4-13	4-26	5-26	6-26	7-28	9-5
GRAMINOIDS																								
Agropyron smithii	1	3	3	7	9	10	1	3	3	4&7	9	10	1	3	3	4 some 8	9	10&3	1	2	3	4&7	10	10-11 &3
A. spicatum																						8	11	12
Aristida longiseta													12	1	3	6	11	11						
Bouteloua gracilis		1	3		10	11			3		11	11	12	1	3	8	10	11		1	3		10	11
Bromus japonicus				9	11	12	1	3	5	9	11	12	1	3	5	9	11	12	1	3	5	9	10	12
B. tectorum				10	11	12				11	12	12				11		12				11	12	12
Carex filifolia															11	12	12					12	12	12
Koeleria cristata	2	4	6	9	11	12	3	4	6	10	11	12	3	4	6	9	11	12	3	3-4	7	10	11	3
Muhlenbergia cuspidata																	8							
Poa pratensis	3	3	6		11	11-12	3	3	6	10	11	11&3	3	3	6	10	11	11&3				10	11	3
P. sandbergii	3	3	6	10	11	12	3	4	6	10-11	11	12	3	3-4	6	10	11	12	3	3	7		11	12
Schedonnardus paniculatus					7	12				6	12						4					8		12
Sporobolus cryptandrus										3										1				
Stipa comata	1	3		10-11	11	12									3	11	12	12		3		11	12	12
S. viridula	1	3	4	10-11	12	12	1	3	4	10-11	12	12	1	3	4	10	12	3	1	3	4	10	12	3
FORBS																								
Achillea millefolium	1	3	5	8-9	12	12	1	3	5	8-9	11	12	1	3	5	8-9	11	12	1	3	5	8	11	12
Allium textile			8	12	12	12			8		12	12			8	12	12	12			8	12	12	12
Androsace occidentalis		11	9	12	12	12			10	12	12	12			10	12	12	12			10	12	12	12
Antennaria sp.			4		12	12						12			8	12	12	12			8		12	12
Arnica sororia		3	8	12	12	12		3	8	12	12	12												
Artemisia ludoviciana				5	5													7			3			
Aster falcatus										4		8				4-5	5	9						
Astragalus crassicarpus														2-3	8		12	12						
A. drummondii				12	12	12										9	12	12						
A. purshii			8										5											
Bahia oppositifolia			3	8	11	12				8	11				3		11	12			3	8	11	12
Besseya wyomingensis		8	10	12	12	12			10	12	12	12		8	10	12	12	12		8	11	12	12	12
Cerastium arvense	1								8		12	12			8		12	12						

---

[1] Codes are given on p. 110.

APPENDIX 16.3. (continued)

SPECIES	CONTROL						LOW						MEDIUM						HIGH					
	4-13	4-26	5-26	6-26	7-26	9-5	4-13	4-26	5-26	6-26	7-28	9-5	4-13	4-26	5-26	6-26	7-28	9-5	4-13	4-26	5-26	6-26	7-28	9-5
FORBS (continued)																								
Camelina microcarpa				9																				
Chenopodium album									3	7	8	10				5	7	11						11
Chorispora tenella		8	9		11	12						11									9	10		11
Crepis sp.															5									
Descurainia sp.				9																				
Erigeron divergens				9	9-10	12	1	3		9	10 8-12	12		2		9	9	12				9	10	12
Gaura coccinea																8								
Grindelia squarrosa				5	5	9				5	6	10				5	6	9	1	3		5	6	9
Hedeoma hispida					12	12																		12
Lactuca serriola					12						10						9	10					5	
Lepidium densiflorum	2	3	6-9	11	12	12			10	11	12	12			10	11	12	12			10	11	12	12
Leucocrinum montanum															11	12	12	12						
Lithospermum ruderale			3	4																				
Lomatium sp.	4	8	9	12	12	12	4	8			12	12	3	8	11	12	12	12						
L. orientale																			4	8	10		12	12
Mammilaria missouriensis			6		12	12		3	6		12	12												
Mertensia oblongifolia															7									
Opuntia fragilis	12	1	5	12	12	12	12	3	5	12	12	12			5	8	12	12		1	5		12	12
O. polyacantha													12	2	3	9	11	12	12	1	5	8	12	12
Orthocarpus luteus										8	12	12					12	12					12	12
Penstemon nitidus			8		12	12																		
Phlox hoodii	4	8	8	12	12	12	3	8		12	12	12		8-9	9	12	12	12						
Plantago patagonica				8	12	12																		
P. spinulosa					12	12					11	12					12	12						12
Polygonum viviparum												11												
Psoralea argophylla																9	11	12						
P. esculenta				9											5		12	12						
Ranunculus glaberrimus							3	4		12	12	12												

APPENDIX 16.3. (continued)

SPECIES	CONTROL 4-13	4-26	5-26	6-26	7-26	9-5	LOW 4-13	4-26	5-26	6-26	7-28	9-5	MEDIUM 4-13	4-26	5-26	6-26	7-28	9-5	HIGH 4-13	4-26	5-26	6-26	7-28	9-5
FORBS (continued)																								
Ratibida columnifera										10	11	11				9	10	11				6	9	
Solidago missouriensis																	7	9						
Sphaeralcea coccinea			4-5	10	11	12				9	12	12			4	9	12	12			4	9	12	12
Taraxacum officinale	1	5	11	12	12	12	1	5	11	12&3	12	12	1		11	12	12	12	1	3	5	12&3	12&3	12&3
Tragopogon dubius	1	3	4	8& 12&3	12&3	12	1	3	5	11&3	12&3	12	1	3	5	11	12&3	12&3						
Vicia americana		3	8	12	12	12				10	12	12			8	10	12	12		2	8	10	12	12
Viola nuttallii															11	12	12	12				12		12
Zygadenus venenosus			7	12	12	12									8	12	12	12			8	12	12	12
HALF-SHRUBS AND SHRUBS																								
Artemisia cana									3	3					3	3	5	8	1	3		3	5	7
A. frigida	1	3	3	3	5	8	1	3	3	3	5	8		2	3	3	5	8	1	3	3	3	5	8
A. tridentata	1	3	3	3	5	9		3		3	5	8	1	3	3	3	5	8	1	3		3	5	6
Atriplex nuttallii			4		9	10													12	2	3	6	9	10
Ceratoides lanata	1	3	6	8	9	10							12	2	5	8	11	11	12	3	5	8	11	11
Xanthocephalum sarothrae					5	9																4		9

SECTION 17

EFFECTS OF LOW-LEVEL SO_2 ON TWO NATIVE LICHEN SPECIES

S. Eversman

ABSTRACT

The objective of this portion of the lichen project has been to determine dose-response curves for lichens subjected to SO_2. *Usnea hirta* (L.) Wigg. and *Parmelia chlorochroa* Tuck. samples showed a statistically significant increase in percentage of plasmolyzed algal cells when exposed to medium and high levels of SO_2 on the ZAPS sites, and a significant decrease in respiration rates at high levels of SO_2. Responses of the lichens to SO_2 at low and medium SO_2 dosages were generally more erratic in 1977 than in 1975 and 1976. Sulfation rates were greater at 50 cm above ground than at 10 cm or 100 cm in plots B, C, and D. The lichen samples at 50 cm exhibited more marked effects from SO_2 than the lichen samples collected from 10 cm and 100 cm.

INTRODUCTION

Summary of 1975-1976 Activities on the ZAPS Sites

The primary objective of this portion of the lichen project has been to determine anatomical and physiological effects of low-level SO_2 on two native lichen species, *Usnea hirta* (L.) Wigg. and *Parmelia chlorochroa* Tuck. A secondary objective has been to attempt comparison of the sensitivity of lichens with that of associated vascular plants, particularly grasses.

I transplanted samples of the two lichen species onto fenceposts in ZAPS Site I in 1975, and in ZAPS Site I and II in 1976. The samples were collected monthly and returned to the laboratory for determination of respiration rate and percentage of plasmolyzed algal cells. Simultaneous collections of the grasses *Agropyron smithii* and *Koeleria cristata* in June, 1975, and June, July, and August, 1976 were made and the respiration rates of the leaves determined.

Significant differences in respiration rates of *Agropyron smithii* samples from plots A and D occur in 1 of 4 readings from ZAPS I, and 1 of 3 readings from ZAPS II, but there is no obvious relationship to length of SO_2 treatment (Appendix 17.1). In 5 of 7 respiration rate determinations of *Koeleria cristata* samples, specimens from plots C and D have a higher (not significant) respiration rate than do samples from plot A, the control. I feel that an effect of SO_2 may be present, but if so, it is subtle.

In 1975 and 1976, the respiration rates of *U. hirta* samples, all collected from about 50 cm above the ground, decreased very significantly ($p < .01$) within 31 days of SO_2 treatment in test plots C and D, ZAPS I and II. The respiration rates of those samples remained significantly lower than those of control samples throughout the test periods (Appendix 17.2). The respiration rates of *U. hirta* samples from plot B decreased, but were not significantly lower than those of control samples until after at least 72 days of treatment (1976, ZAPS I). The rate did not remain statistically lower consistently throughout the test period on plot B, ZAPS II, 1976.

Direct visual microscopic observations of *U. hirta* (i.e., counting plasmolyzed algal cells) appeared to be a sensitive method of detecting adverse SO_2 effects and gave reliable results (Appendix 17.3). A very significant ($P < .01$) increase in number of plasmolyzed bleached algal cells appeared within 31 days in *U. hirta* samples from plots B, C, and D, and remained significantly higher than the controls throughout the test periods. More than 90% of the algal cells were plasmolyzed in the *U. hirta* samples from plots C and D within 30 days of exposure; samples from plots C and D were nearly identical in percentage of plasmolyzed cells. During 156 days of SO_2 treatment in 1976, the percentages of bleached, plasmolyzed algal cells from *U. hirta* samples from ZAPS I and II, plots B, remained significantly higher than those of samples from plot A and lower than those of samples from plots C and D.

Parmelia chlorochroa which was transplanted to the base of fenceposts on the sulfation treatment plots in 1975 appeared to be much less sensitive to SO_2 than *Usnea hirta*. The respiration rates of samples collected in 1975 did not vary significantly during 110 days of SO_2 treatments (Appendix 17.4), and the plasmolysis rates increased significantly only on plots C and D after 60 days of SO_2 exposure (Appendix 17.5).

More than species differences, the vertical position of the samples on the fenceposts in the plots may have been responsible for the different responses of *U. hirta* and *P. chlorochroa*. Elevating *P. chlorochroa*, by tying thalli on empty ponderosa pine branches which were then wired onto the posts adjacent the *U. hirta*, gave the results tabulated in Appendix 17.4. Elevated samples from Plot D had respiration rates significantly less ($P < .01$ level) than those of control samples at 23 and 60 days. By 60 days, elevated *P. chlorochroa* samples from B and C also had significantly lower respiration rates than control samples, and the algal cell plasmolysis increased significantly (Appendix 17.5).

The results led to experiments designed to investigate vertical differences in SO_2 on the ZAPS I test plots, and to attempt to more closely correlate SO_2 dosage with lichen responses.

1977 Activities on ZAPS Sites

The SO_2 monitoring system on the ZAPS plots had samplers 35 cm above ground level. The lichen samples that showed the most obvious, and statistically significant, responses to SO_2 were about 50 cm above ground. *P. chlorochroa* samples on the ground were not exhibiting SO_2 damage as convincingly as anticipated. It appeared from the lichen information from 1975 and 1976 that either less SO_2 was reaching lichens on the ground or that lichens on the ground were less susceptible to SO_2. Sulfation plates, *U. hirta* samples, and *P. chlorochroa* samples were placed adjacent each other at different heights to obtain more precise dose-response data in 1977.

METHODS AND MATERIALS

I obtained sulfation plates from the Air Quality Bureau, Montana Department of Health, Helena. Their methods of preparing and analyzing these plates are presented in Appendix 17.6.

The locations of fenceposts on each ZAPS I and II test plot are shown in Figure 17.1. Sulfation plates were wired on poles 1, 3, and 4, at 100, 50, and 10 cm above the ground (9 plates/plot). The plates faced north or northeast in each case because of the ridges on the fence posts. The dates of exposure of each set of plates were: 1) June 22-July 6, 2) July 6-July 19, 3) July 19-August 3, 4) August 3-August 17. Ponderosa pine branches containing *U. hirta* or *P. chlorochroa* samples were wired adjacent the sulfation plates. *U. hirta* from ZAPS I was collected with each change of sulfation plates (every two weeks). *U. hirta* from ZAPS II and *P. chlorochroa* were collected monthly. Collection dates from 1977 (also 1975 and 1976) are in Appendix 17.7.

Respiration rate was measured in a Gilson respirometer for 250 mg of sample material for 1 hour at 20°C.

To determine percentage of plasmolyzed algal cells, I scraped pieces of several thalli into 3 separate water drops on a microscope slide, then counted 100 algal cells in each drop, recording the number of plasmolyzed algal cells.

Changes in chlorophyll absorption were determined by making two or three 3-ml extractions with boiling methanol from 300 mg lichen samples, then adding methanol to make 10 ml. Absorbance was read at 665 nm on a Beckman DU Spectrophotometer.

RESULTS AND DISCUSSION

Individual sulfation plate results appear in Table 17.1. The results from the Montana Department of Health Laboratory were reported as

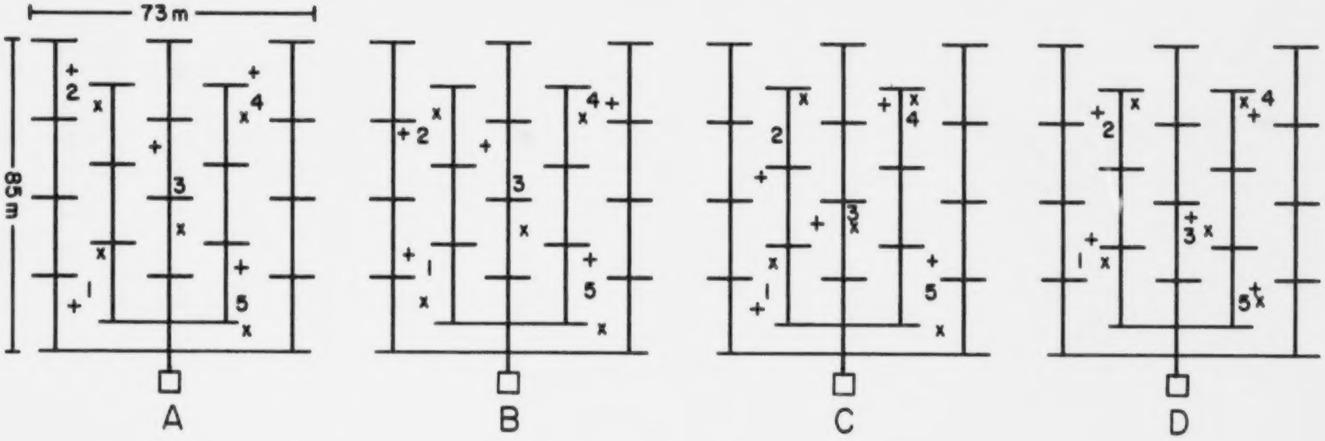

73m
85m
1
2
3
4
5
A
B
C
D

Figure 17.1. Locations of fenceposts on ZAPS I and II sites. + = ZAPS I fenceposts; x = ZAPS II fenceposts.

TABLE 17.1 SULFATION, PPHM SO_2 BY INDIVIDUAL LOCATION, 1977 (24 JUNE - 17 AUGUST)

Sample Identification Code:
A, B, C, D = ZAP I plot
1, 2, 3 = fence post
a, b, c = height: a = 100 cm, b = 50 cm, c = 10 cm
1, 2, 3, 4 = collection date: 1 = 7-3-77, 2 = 7-19-77, 3 = 8-3-77, 4 = 8-17-77

Sample Identification	SO_2 pphm	Sample Identification	SO_2 pphm	Sample Identification	SO_2 pphm
A1a1	0.315	A1b1	0.280	A1c1	0.035
A3a1	0.175	A3b1	0.035	A3c1	0.000
A4a1	0.210	A4b1	0.105	A4c1	0.070
A1a2	0.105	A1b2	0.000	A1c2	0.000
A3a2	0.000	A3b2	0.000	A3c2	0.035
A4a2	0.105	A4b2	0.000	A4c2	0.000
A1a3	0.700	A1b3	0.420	A1c3	0.380
A3a3	0.700	A3b3	0.490	A3c3	0.070
A4a3	0.150	A4b3	0.525	A4c3	0.420
A1a4	0.525	A1b4	0.058	A1c4	0.000
A3a4	0.140	A3b4	0.140	A3c4	0.035
A4a4	0.105	A4b4	0.350	A4c4	0.140
B1a1	1.365	B1b1	1.540	B1c1	0.630
B3a1	1.330	B3b1	2.100	B3c1	1.015
B4a1	0.875	B4b1	0.450	B4c1	---
B1a2	1.925	B1b2	2.065	B1c2	0.805
B3a2	1.680	B3b2	2.065	B3c2	0.735
B4a2	1.155	B4b2	1.400	B4c2	---
B1a3	2.520	B1b3	3.010	B1c3	1.190
B3a3	1.960	B3b3	2.800	B3c3	1.155
B4a3	1.785	B4b3	1.400	B4c3	0.980
B1a4	2.135	B1b4	1.575	B1c4	1.225
B3a4	1.785	B3b4	1.750	B3c4	1.085
B4a4	1.750	B4b4	1.225	B4c4	1.050
C1a1	2.100	C1b1	1.720	C1c1	2.065
C3a1	3.640	C3b1	1.960	C3c1	0.805
C4a1	--	C4b1	3.960	C4c1	0.805
C1a2	2.980	C1b2	3.610	C1c2	1.680
C3a2	3.200	C3b2	4.170	C3c2	1.610
C4a2	2.400	C4b2	2.730	C4c2	1.400

TABLE 17.1 (Cont.)

Sample Identification	SO_2 pphm	Sample Identification	SO_2 pphm	Sample Identification	SO_2 pphm
C1a3	3.200	C1b3	4.400	C1c3	2.275
C3a3	2.800	C3b3	2.500	C3c3	1.960
C4a3	3.590	C4b3	3.290	C4c3	1.295
C1a4	3.500	C1b4	3.300	C1c4	1.110
C3a4	2.800	C3b4	3.500	C3c4	1.860
C4a4	3.360	C4b4	3.850	C4c4	1.190
D1a1	7.245	D1b1	10.325	D1c1	---
D3a1	5.250	D3b1	14.210	D3c1	7.00
D4a1	6.510	D4b1	5.635	D4c1	---
D1a2	5.390	D1b2	6.650	D1c2	3.920
D3a2	5.150	D3b2	12.950	D3c2	5.530
D4a2	4.170	D4b2	5.640	D4c2	2.170
D1a3	5.530	D1b3	7.210	D1c3	3.190
D3a3	5.220	D3b3	13.370	D3c3	4.130
D4a3	3.400	D4b3	3.610	D4c3	2.170
D1a4	5.360	D1b4	10.610	D1c4	3.750
D3a4	6.760	D3b4	17.780	D3c4	3.640
D4a4	4.800	D4b4	4.450	D4c4	3.540

mg SO_3/100 cm^2/day. I used a conversion factor of.035 to convert those units to ppm (Corning Laboratories), then multiplied by 100 for pphm. Table 17.2 gives the means of SO_2 in pphm, by heights, 100, 50, and 10 cm, for June 22-August 17, 1977, and results of one-way analysis of variance. Only ZAPS I, plot A showed no significant differences between the three heights surveyed.

On plots B, C, and D, the amounts of SO_2 received by the sulfation plates at 10 cm above the ground were significantly less than the SO_2 amounts received by the higher plates. In plots B, C, and D, the greatest amounts of SO_2 occurred at the 50 cm height.

There were no significant differences in sulfation rate means (4 collections, each about 14 days of exposure) at the 50 cm heights between plots A and B, and B and C; and at the 100 cm height on plots B and C, for the 56 days of monitoring with the sulfation plates.

Some of the lichen observations reflect these results. *U. hirta* samples from plot D generally had significantly lower respiration rates than samples from other plots (Figures 17.2-5, Table 17.3). However, the respiration rates of *U. hirta* samples from plots B and C fluctuated considerably, especially in relation to samples from plot A.

Determination of absorbance spectra of chlorophyll extracts of *U. hirta* samples (Table 17.4) gave relative results similar to those of respiration rate determination.

The results obtained from cell plasmolysis counts of *U. hirta* are in Table 17.5. Again, the results are less clear-cut than those obtained previously, but are consistently significant between samples from plot A and plots C and D. An expected significant difference between *U. hirta* samples from plots A and B did not appear in 56 days. Finished sets of plasmolysis readings for *U. hirta* and *P. chlorochroa* for 1977 are not yet complete.

Fairly consistent significant differences in respiration rate of elevated *P. chlorochroa* samples occurred between samples from plot A, and from plots C and D (Figures 17.2-5, Table 17.6). Significant differences did not occur between samples from plots A and B. Only samples from plot D exhibited consistent significantly lower absorbance by chlorophyll extracts after 84 days of treatment (Table 17.7).

Counts of plasmolyzed algal cells in *P. chlorochroa* showed no significant differences in samples from all plots after 27 days of SO_2 treatment (Table 17.8). After 56 days of exposure, samples from plot D have a significantly higher percentage of plasmolyzed algal cells than samples from plot A. Samples from plots B and C are intermediate.

TABLE 17.2. STATISTICAL COMPARISONS OF SO_2 RATES OBTAINED FROM SULFATION PLATES ON POLES 1, 3, 4; ZAPS I, 1977

Plot	Height cm	$\bar{X}$ * pphm	CI .95	ANOVA †	F
A	100	0.27	0.15	$\underline{A_{100cm}A_{50cm}A_{10cm}}$	2.37
	50	0.17	0.13		
	10	0.09	0.08		
B	100	1.69	0.29	$\underline{B_{50}B_{100}}B_{10}$	7.88
	50	1.78	0.44		
	10	0.99	0.14		
C	100	2.96	0.32	$\underline{C_{50}C_{100}}C_{10}$	22.60
	50	3.25	0.55		
	10	1.60	0.32		
D	100	5.40	0.68	$D_{50}\underline{D_{100}D_{10}}$	10.98
	50	9.37	2.86		
	10	3.90	1.04		
				$A_{100}B_{100}C_{100}D_{100}$	136.54
				$\underline{A_{50}B_{50}}C_{50}D_{50}$	36.05
				$A_{10}\underline{B_{10}C_{10}}D_{10}$	50.10

* Mean pphm SO_2 from 12 samples with ±.95 confidence interval, 24 June - 17 August 1977.

† Results of one-way analysis of variance. Locations with significantly different sulfation rates ($P < .05$) do not share the same underline.

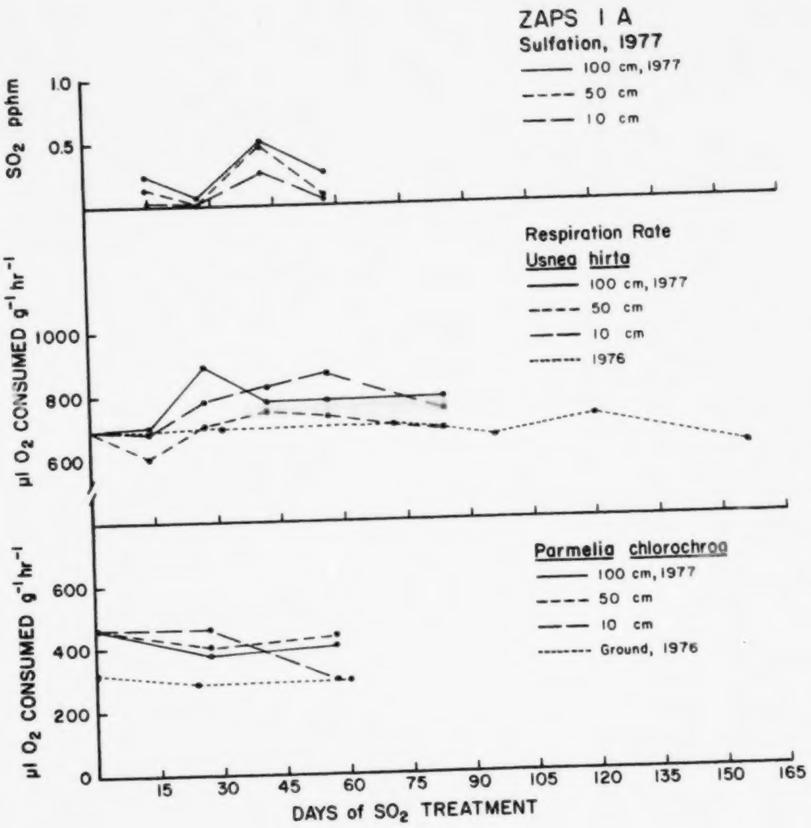


Figure 17.2. Sulfation rate (pphm SO_2) 22 June - 17 August, 1977; respiration rates of *Usnea hirta* and *Parmelia chlorochroa*, 1976 and 1977; ZAPS I A.

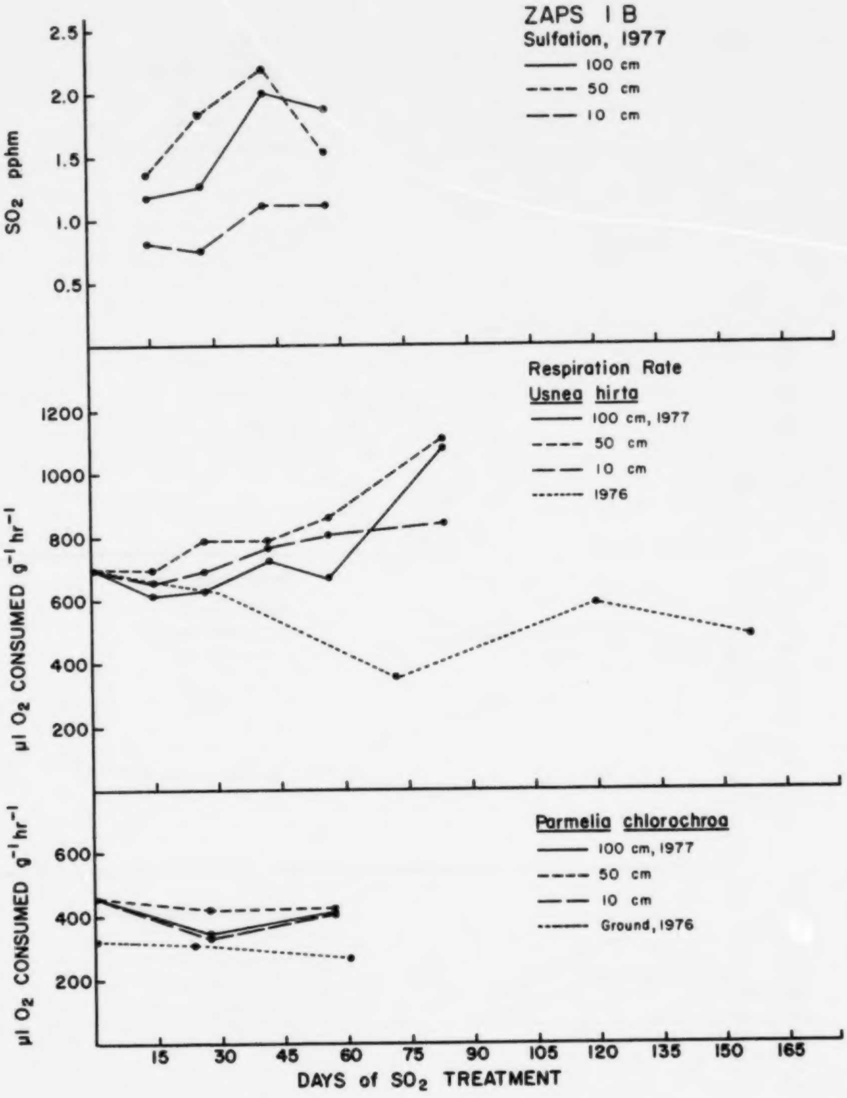


Figure 17.3. Sulfation rate (pphm SO_2) 22 June - 17 August, 1977; respiration rates of *Usnea hirta* and *Parmelia chlorochroa*, 1976 and 1977; ZAPS I B.

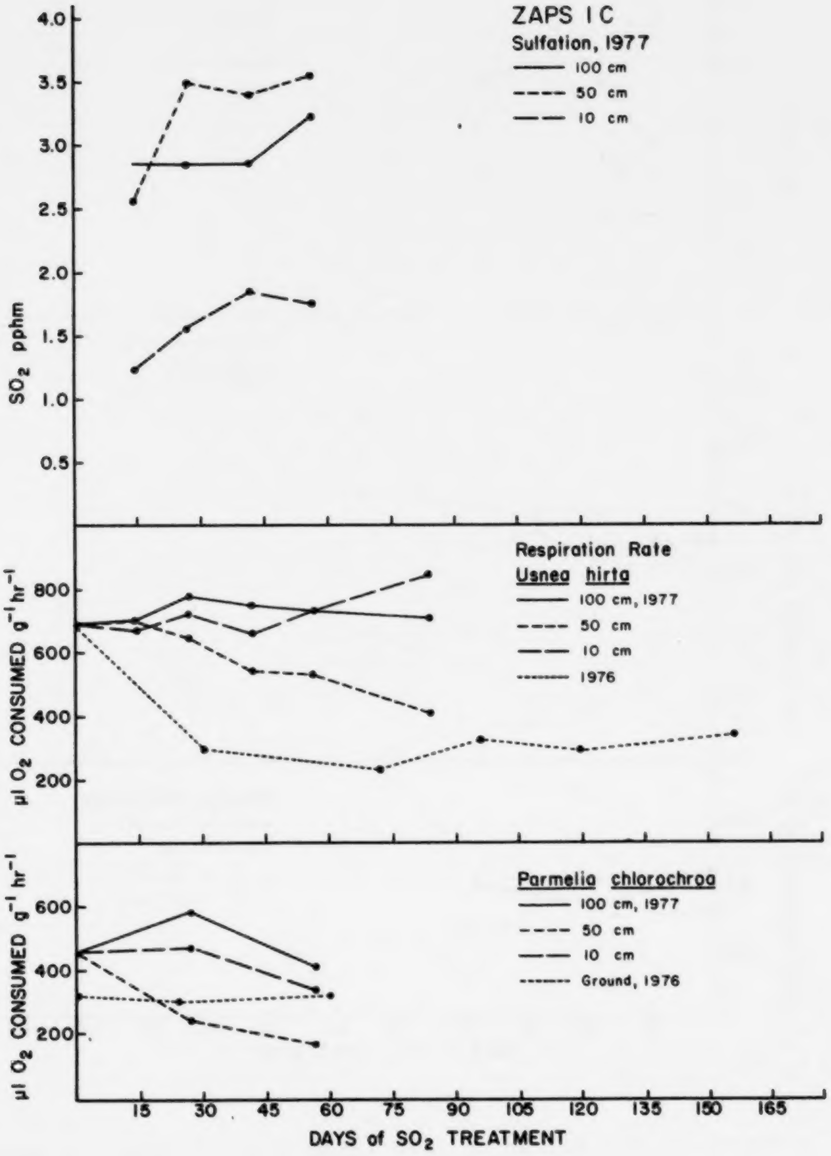


Figure 17.4. Sulfation rate (pphm SO$_2$) 22 June - 17 August, 1977; respiration rates of *Usnea hirta* and *Parmelia chlorochroa*, 1976 and 1977; ZAPS I C.

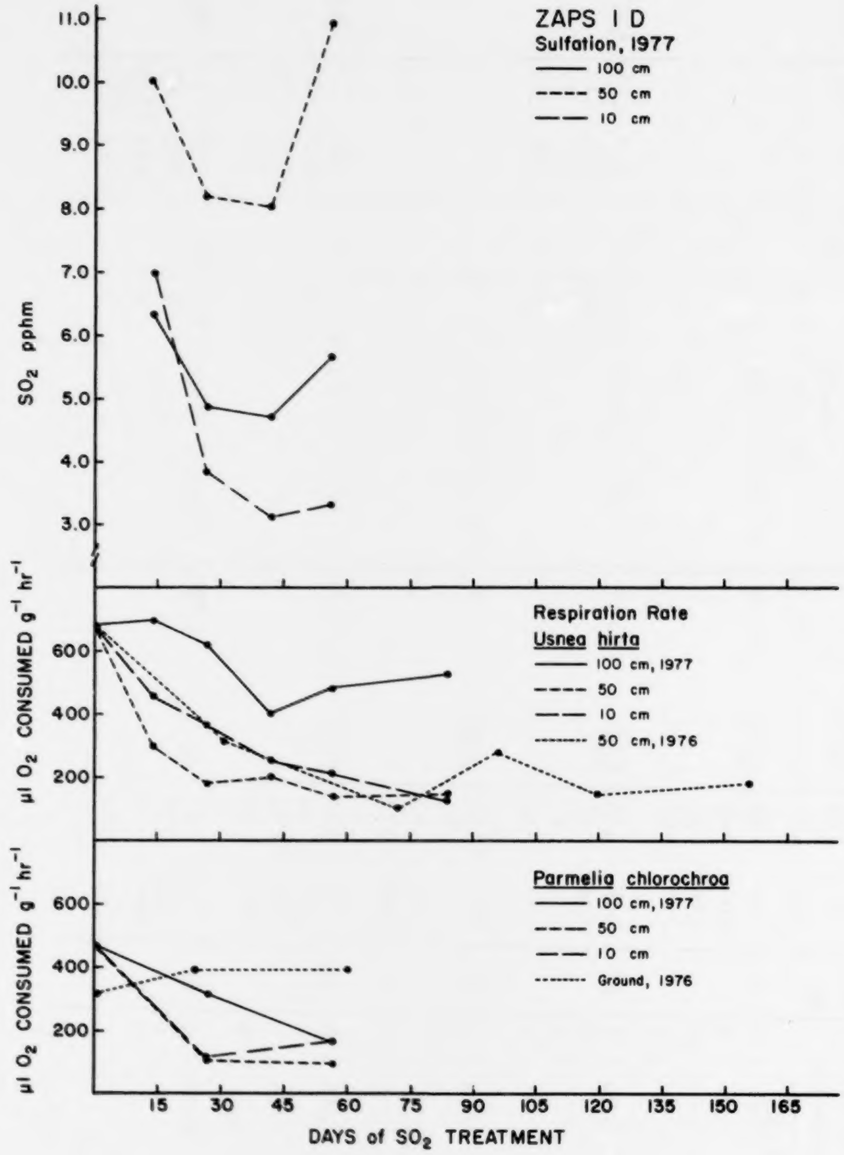


Figure 17.5. Sulfation rate (pphm SO_2) 22 June - 17 August, 1977; respiration rates of *Usnea hirta* and *Parmelia chlorochroa*, 1976 and 1977; ZAPS I D.

TABLE 17.3 RESPIRATION FATES OF *USNEA HIRTA* COLLECTED FROM ZAPS I AND ZAPS II, 1977

Collection Date, Sample Location		Days SO_2	$\bar{X}$*	CI	ANOVA†	F	Collection Date, Sample Location		Days SO_2	$\bar{X}$*	CI	ANOVA†	F
100 cm							100 cm						
7-06-77 ZAPS I	A	14	704	49	ADCB	4.26	no collection						
	B		607	48									
	C		696	56									
	D		704	89									
7-19-77 ZAPS I	A	27	887	146	ACBD	9.46	7-19-77 ZAPS II	A	27	819	191	BACD	2.16
	B		636	97				B		863	413		
	C		775	45				C		787	87		
	D		623	51				D		646	293		
8-03-77 ZAPS I	A	42	786	121	ACBD	4.32	no collection						
	B		724	73									
	C		753	67									
	D		602	123									
8-17-77 ZAPS I	A	56	788	111	ACBD	17.32	8-17-77 ZAPS II	A	56	748	27	CABD	39.09
	B		666	64				B		637	176		
	C		736	46				C		795	95		
	D		481	96				D		419	67		
9-14-77 ZAPS I	A	84	791	183	BACD	3.78	9-14-77 ZAPS II	A	84	919	70	ABDC	75.79
	B		1089	818				B		802	65		
	C		715	36				C		195	1154		
	D		530	117				D		315	703		

TABLE 17.3 (Cont.)

Collection Date, Sample Location	Days SO_2	$\bar{X}^*$	CI	ANOVA†	F	Collection Date, Sample Location	Days SO_2	$\bar{X}^*$	CI	ANOVA†	F
50 cm						50 cm					
7-06-77 ZAPS I A	14	602	58	BCAD	12.86	no collection					
B		698	91								
C		686	103								
D		312	217								
7-19-77 ZAPS I A	27	704	43	BACD	98.91	7-19-77 ZAPS II A	27	720	80	CBAD	56.20
B		790	50			B		737	219		
C		649	148			C		740	194		
D		191	90			D		184	85		
8-03-77 ZAPS I A	42	757	93	BACD	28.37	no collection					
B		788	84								
C		544	184								
D		202	135								
8-17-77 ZAPS I A	56	738	71	BACD	41.17	8-17-77 ZAPS II A	56	737	55	ABCD	100.49
B		855	122			B		624	84		
C		537	116			C		612	199		
D		148	155			D		106	89		
9-14-77 ZAPS I A	84	684	38	BACD	38.36	9-14-77 ZAPS II A	84	852	582	BACD	15.61
B		1112	291			B		878	105		
C		414	351			C		627	275		
D		159	166			D		97	72		

TABLE 17.3 (Cont.)

Collection Date, Sample Location	Days SO_2	$\bar{X}$[*]	CI	ANOVA[†]	F	Collection Date, Sample Location	Days SO_2	$\bar{X}$[*]	CI	ANOVA[†]	F
10 cm						10 cm					
7-06-77 ZAPS I A	14	693	109	ACBD	7.12	no collection					
B		663	37								
C		680	105								
D		461	152								
7-19-77 ZAPS I A	27	782	441	ACBD	9.06	7-19-77 ZAPS II A	27	591	109	CBAD	24.66
B		694	66			B		729	120		
C		729	130			C		739	95		
D		363	251			D		212	164		
8-03-77 ZAPS I A	42	832	31	ABCD	29.87	no collection					
B		729	50								
C		668	71								
D		255	165								
8-17-77 ZAPS I A	56	870	67	ABCD	71.99	8-17-77 ZAPS II A	56	628	189	BCAD	60.12
B		805	82			B		801	191		
C		734	113			C		681	102		
D		215	136			D		145	142		
9-14-77 ZAPS I A	84	756	1253	BAD	22.88	9-14-77 ZAPS II A	84	836	127	ABCD	61.06
B		844	477			B		774	207		
C		-	-			C		449	839		
D		136	47			D		132	902		

[*] Respiration rates are expressed as µl oxygen consumed $g\text{-}hr^{-1}$, means of 2-6 samples with ±.95 confidence interval (CI).

[†] Results of one-way analysis of variance. Locations with significantly different respiration rates $(P < .05)$ do not share the same underline.

TABLE 17.4. RELATIVE ABSORBANCE AT 665 nm LIGHT OF CHLOROPHYLL EXTRACTS FROM *USNEA HIRTA* COLLECTED FROM ZAPS I AND THE TRANSPLANT SOURCE (EOC: EAST OTTER CREEK), 1977

Collection date, Sample Location		Days SO_2	$\bar{\bar{X}}$*	CI .95	ANOVA†	F
4-27-77 EOC transplant source		0	.225	.120		
ZAPS I (overwintering)	A	(1976)	.147	.090	$A\underline{BCD}$	32.73
	B		.039	.034		
	C		.014	.013		
	D		.014	.004		
7-19-77						
100 cm	A	27	.168	.116	$\underline{BCDA}$	0.71
	B		.217	.004		
	C		.202	.017		
	D		.183	.026		
50 cm	A	27	.266	.267	$\underline{ACBD}$	9.42
	B		.174	.009		
	C		.202	.017		
	D		.038	.004		
10 cm	A	27	.175	.202	$\underline{BAC}$	1.95
	B		.217	.026		
	C		.140	.034		
8-17-77						
100 cm	A	56	.236	.013		
	C		.119	.191		
	D		.049	.114		
50 cm	A	56	.200	.1025		
	B		.209	.140		
	D		.044	.089		
10 cm	A		.331	1.436		
	D		.027	.076		9.63
				$\underline{A^{‡}_{10}\ A_{100}\ B_{50}\ A_{50}}\ C_{100}\ D_{100}\ D_{50}\ D_{10}$		
6-23-77 ZAPS II	A	(1976)	.163	.129		
8-16-77 EOC			.211	.086		
9-17-77 ZAPS II	A100	84	.188	.077		
	A50	84	.195	.065		
	A10	84	.130	.026		

* Means of relative absorbance readings of 3 extracts with ± .95 confidence interval.

† Results of one-way analysis of variance. Locations with significantly different absorption means do not share the same underline.

‡ A_{10} *Usnea hirta* sample from 10 cm height, treatment plot A; etc.

TABLE 17.5. PERCENTAGE OF PLASMOLYSIS OF ALGAL CELLS OF *USNEA HIRTA* COLLECTED FROM ZAPS I AND ZAPS II AND THE TRANSPLANT SOURCES (EOC: EAST OTTER CREEK), 1977

Collection Date	Sample Location		Days SO_2	$\bar{X}$*	$CI_{.95}$	ANOVA†	F
3-26-77	EOC		0	3.0	6.5		
7-19-77	ZAPS I	A50	27	3.7	1.3	ABCD	211.49
		B50		30.0	24.5		
		C50		67.0	2.6		
		D50		99.3	3.01		
8-17-77	ZAPS I	A100	56	13.3	9.9		
		B100		16.3	12.5		
		B100		24.3	19.9		
		B50		24.7	19.9		
		B10		30.7	27.3		
		C100		41.3	54.7		
		C10		62.0	5.0		
		D10		85.3	14.9		
		C50		97.7	7.5		
		D50		98.3	7.5		
		$\underline{A_{100}\ B_{100}\ B_{100}\ B_{50}}\ \underline{B_{10}\ C_{100}}\ C_{10}\ \underline{D_{10}\ C_{50}\ D_{50}}$					88.75

* Means of counts of 3 samples with ± .95 confidence interval.

† Results of one-way analysis of variance. Locations with significantly different plasmolysis determinations do not share the same underline.

A50 = *Usnea* samples collected from 50 cm height, treatment plot A, etc.

TABLE 17.6. STATISTICAL ANALYSIS OF RESPIRATION RATES OF *PARMELIA CHLOROCHROA* COLLECTED FROM ZAPS I AND THE TRANSPLANT SOURCE (KLUVER WEST), 1977

Collection Date Sample Location		Days SO_2	$\bar{X}$*	CI	ANOVA†	F
6-20-77	KLW	0	460	122		
7-19-77						
100 cm ZAPS	A	27	390	51	CABD	17.82
	B		368	60		
	C		585	137		
	D		321	75		
50 cm ZAPS	A		408	115	BACD	30.32
	B		424	67		
	C		240	148		
	D		114	7		
10 cm ZAPS	A		461	197	CABD	13.70
	B		359	126		
	C		475	126		
	D		119	85		
8-17-77						
100 cm ZAPS	A	56	403	110	BCAD	25.75
	B		419	49		
	C		407	22		
	D		163	95		
50 cm ZAPS	A		439	33	ABCD	103.01
	B		430	48		
	C		167	42		
	D		100	44		
10 cm ZAPS	A		297	37	BCAD	13.24
	B		412	62		
	C		335	165		
	D		165	36		

* Respiration rates are expressed as μl oxygen consumed g-hr^{-1}, means of 2-6 samples with ±.95 confidence interval (CI).

†Results of one-way analysis of variance. Locations with significantly different respiration rates ($P < .05$) do not share the same underline.

TABLE 17.7 RELATIVE ABSORBANCE AT 655 nm LIGHT OF CHLOROPHYLL EXTRACTS FROM *PARMELIA CHLOROCHROA* COLLECTED FROM ZAPS I, 1977

Collection Date Sample Location		$\bar{X}$*	CI	ANOVA †	F
8-17-77					
100 cm	A	.118	.065	$\underline{\text{CAB}}\text{D}$ $\phantom{\text{C}}\underline{\phantom{\text{ABD}}}$	6.90
	B	.091	.004		
	C	.142	.056		
	D	.007	.039		
50 cm	A	.065	.003	$\text{B}\underline{\text{AC}}\text{D}$	64.68
	B	.084	.017		
	C	.060	.013		
	D	.031	0		
10 cm	A	.118	.065	$\underline{\text{AB}}\text{CD}$	29.32
	B	.116	.003		
	C	.006	.017		
	D	.034	.004		
9-14-77	A native	.039	.025		

* Means of relative absorbance readings of 3 extracts with ± .95 confidence interval (CI).

† Results of one-way analysis of variance. Locations with significantly different ($P < .05$) absorbance means do not share the same underline.

TABLE 17.8. PERCENTAGE OF PLASMOLYSIS OF ALGAL CELLS OF *PARMELIA CHLOROCHROA* COLLECTED FROM ZAPS I, 1977

Collection Date	Sample Location	Days SO_2	$\bar{X}$[*]	CI	ANOVA[†]	F
7-19-77	A100[‡]	27	9.0	12.5	DBCA	2.79
	B50		34.7	33.1		
	B10		12.7	19.9		
	C50		8.0	21.5		
	D10		41.7	109.4		
	D50		53.0	19.8		
8-17-77	A100	56	12.0	5.0		4.80
	A10		8.0	5.0		
	B50		24.7	49.7		
	C100		13.0	12.5		
	C50		44.3	29.8		
	D100		52.0	42.3		
	D10		51.0	69.6		
					D_{100} D_{10} C_{50} B_{50} C_{100} A_{100} A_{10}	

*Means of counts of 3 samples with ± .95 confidence interval (CI).

†Results of one-way analysis of variance. Locations with significantly different plasmolysis determinations do not share the same underline.

‡A100 = *Parmelia* samples collected from 100 cm height, plot A; etc.

As in 1975 and 1976, we collected samples of *U. hirta* and *P. chlorochroa* from many field sites to compare with samples from the ZAPS A plots. Analysis of variance of respiration rate data of *U. hirta* from 1977 have shown no significant differences in respiration rate from collection date 1 (July 6) to collection date 5 (September 14); these rates are not significantly different from the rates of simultaneously collected samples from the transplant source (East Otter Creek)and other ponderosa pine sites at any time. Analyses of variance have also consistently indicated no differences in *P. chlorochroa* samples between ZAPS and field sites. Therefore, I have used samples from ZAPS A plot as controls.

Analysis of variance of respiration rate of *U. hirta* samples collected from ZAPS I plot B from June through September, 1977 indicates a gradual significant increase ($P < .01$) on all heights from the first to the last collection; the rise in rate is progessive by date. An interpretation of this phenomenon appears below. The analysis of variance of samples from plots C and D shows a significant decrease, compared with control samples, within 14 days, then no significant respiration rate decrease from July 3 to September 14. Damage by SO_2 at the levels of plots C and D, as measured by respiration rate and plasmolysis of algal cells, is acute and immediate in *U. hirta*.

Some of the lichen samples in 1977 and the grass samples in 1976 from plots B, C, and D exhibited increases in respiration rates compared with samples from plot A. This behavior was different from the patterns established in 1975 and 1976. I have interpreted this phenomenon as an effect of low-level SO_2 stress, previously described by Baddeley *et al*, (1971) and Puckett *et al*, (1973). These workers consider an increase in respiration rate to be an observable reaction of plants to stress, similar to a wounding reaction. Larger and/or longer amounts of SO_2 exposure decrease respiratory activity, as in plots B, C, and D, 1976 and 1977.

Parmelia chlorochroa at elevated heights, exhibited effects of SO_2 exposure slightly less pronounced than those of *U. hirta*. The mean percentage of plasmolyzed cells in *P. chlorochroa* samples did not exceed 72%. The mean percentage of plasmolyzed cells in *U. hirta* reached nearly 100% in 30-50 days in plots C and D. Observing the thalli microscopically is a tedious method of quantifying SO_2 effects, and it should be done by one person to minimize subjective individual factors. However, I feel it is a reliable and necessary method for detecting SO_2 effects. Since one must handle the material closely, the external visible effects--thallus crumbling and bleaching--are also more readily noted.

Another microscopically visible bit of evidence of damage by SO_2 is the change in location of the bacterial populations associated with the lichens, particularly with *P. chlorochroa*. In fresh mounts of healthy lichens, many bacteria are visible in the water on the slide. As the lichen is exposed to SO_2 and the integrity of the lichen thallus decreases, more bacteria become attached to the fungal hyphae and sometimes to the algal cells; so many that sometimes it is difficult to identify an algal cell. I have not yet determined a method to quantify or identify a reason for the change in bacterial position. However, other researchers (Puckett, et al. 1974; Malhotra and Hocking, 1976) and I have found an increase in permeability of the lichen cells with deterioration of cell condition, which may be related to the bacteria observation. The marked differences in resident bacterial populations in *P. chlorochroa* probably also contribute to the increased respiration rate found especially in samples collected from the ground.

Statistical analysis of other measurements is not yet complete.

CONCLUSIONS

Usnea hirta seems to be slightly more sensitive to SO_2 than *P. chlorochroa*. The respiration rates of *U. hirta* decrease more consistently and the plasmolysis percentage increases more rapidly and to a greater degree than those of *P. chlorochroa*. *U. hirta*, normally located on tree trunks and branches, is more exposed to airborne SO_2 than *P. chlorochroa* and may be more useful as an air quality indicator.

Direct microscopic observation of *P. chlorochroa* is the most reliable method of determining SO_2 effects on this species. Not only can one determine plasmolysis rate, but the behavior of the bacterial populations, and the general thallus condition can also be observed.

REFERENCES

Baddeley, M. S., B. W. Ferry, and E. J. Finegan. 1971. The Effects of Sulphur Dioxide on Lichen Respiration. Lichenologist, 5:283-291.

Malhotra, S.S. and D. Hocking. 1976. Biochemical and Cytological Effects of Sulphur Dioxide on Plant Metabolism. New Phytologist, 76:227-237.

Puckett,K. J., E. Nieboer, W. P. Flora, and D. H. S. Richardson. 1973. Sulphur Dioxide: Its Effects on Photosynthetic ^{14}C Fixation in Lichens and Suggested Mechanisms of Phototoxicity. New Phytologist, 72: 141-154.

Puckett, K. J., D. H. S. Richardson, W. P. Flora, and E. Nieboer. 1974. Photosynthetic ^{14}C Fixation by the Lichen *Umbilicaria muhlenbergii* (Ach). Tuck. Following Short Exposure to Aqueous Sulphur Dioxide. New Phytologist, 73:1183-1192.

APPENDIX 17.1 RESPIRATION RATES OF GRASSES *AGROPYRON SMITHII* AND *KOELERIA CRISTATA* COLLECTED FROM ZAPS I AND ZAPS II, 1975-76

Agropyron smithii Collection Date, Sample Location			Days SO_2	$\bar{X}$[*]	CI	ANOVA[†]	F
6-26-75	ZAPS I	A	33	936	59	ACDB	6.63
		B		761	89		
		C		858	55		
		D		805	147		
6-23-76	ZAPS I	A	72	646	470	ABCD	0.73
		B		736	431		
		C		520	263		
		D		610	584		
	ZAPS II	A		579	74	BACD	31.55
		B		699	174		
		C		551	83		
		D		349	85		
7-17-76	ZAPS I	A	93	355	159	ABDC	2.19
		B		231	224		
		C		197	159		
		D		212	526		
	ZAPS II	A		335	94	CABD	0.65
		B		328	297		
		C		366	405		
		D		249	136		
8-9-76	ZAPS I	A	119	145	32	CDBA	3.12
		B		177	40		
		C		239	72		
		D		197	168		
	ZAPS II	A		312	94	DCAB	20.63
		B		107	80		
		C		394	125		
		D		530	211		

APPENDIX 17.1 (Cont.)

Koeleria cristata Collection Date, Sample Location			Days SO	$\bar{X}$*	CI	ANOVA†	F
6-26-75	ZAPS I	A	33	554	279	$\underline{\text{CBDA}}$	0.67
		B		637	149		
		C		732	196		
		D		635	386		
6-23-76	ZAPS I	A	72	702	126	$\text{DC}\underline{\text{BA}}$	12.76
		B		706	255		
		C		893	260		
		D		1045	104		
	ZAPS II	A		728	6	$\underline{\text{DABC}}$	2.01
		B		617	334		
		C		563	298		
		D		759	284		
7-17-76	ZAPS I	A	96	506	9	$\underline{\text{DBCA}}$	6.92
		B		587	324		
		C		560	178		
		D		827	282		
	ZAPS II	A		247	22	DCBA	9.28
		B		411	187		
		C		430	79		
		D		525	256		
8-9-76	ZAPS I	A	119	127	87	$\underline{\text{BCAD}}$	2.08
		B		164	48		
		C		146	72		
		D		108	78		
	ZAPS II	A		147	63	$\underline{\text{DCBA}}$	19.75
		B		226	112		
		C		375	103		
		D		332	115		

---

* Respiration rates are expressed as μl oxygen consumed g-hr^{-1}, means of 3 samples with ±.95 confidence interval (CI).

† Results of one-way analysis of variance. Locations with significantly different respiration rates (P < .05) do not share the same underline.

APPENDIX 17.2 RESPIRATION RATES OF *USNEA HIRTA* COLLECTED FROM ZAPS I AND ZAPS II, 1975-1976

Collection Date Sample Location			Days SO_2	$\bar{X}^*$	CI	ANOVA†	F
5-13-75	Transplant Source		0	719	37		
6-25-75	ZAPS I	A	33	817	45	ABCD	35.82
		B		746	12		
		C		472	353		
		D		54	56		
7-10-75	ZAPS I	A	67	686	96	ABCD	16.13
		B		599	93		
		C		336	220		
		D		66	95		
9-11-75	ZAPS I	A	110	709	137	ABCD	22.38
		B		499	159		
		C		355	170		
		D		4	16		
5-13-76	ZAPS I	A	31	711	116	ABDC	9.96
		B		618	175		
		C		290	192		
		D		325	247		
6-23-76	ZAPS I	A	72	704	176	ABCD	34.62
		B		359	194		
		C		236	206		
		D		115	166		
7-17-76	ZAPS I	A	96	670	256	ABCD	19.33
		B		473	97		
		C		323	97		
		D		244	229		
8-9-76	ZAPS I	A	119	721	45	ABCD	58.14
		B		556	209		
		C		298	182		
		D		150	72		
9-15-76	ZAPS I	A	156	631	52	ABCD	61.81
		B		487	107		
		C		340	99		
		D		142	169		

APPENDIX 17.2 (Cont.)

Collection Date, Sample Location				Days SO_2	$\bar{X}$*	CI	ANOVA†	F
5-13-76	ZAPS	II	A	31	800	154	$\mathrm{\underline{AB}CD}$	23.61
			B		755	92		
			C		438	278		
			D		264	114		
6-23-76	ZAPS	II	A	72	555	85	$\mathrm{\underline{A\underline{B}}\underline{C}D}$	35.87
			B		476	191		
			C		263	119		
			D		169	95		
7-17-76	ZAPS	II	A	96	643	95	ABCD	59.24
			B		479	214		
			C		264	120		
			D		98	52		
8-9-76	ZAPS	II	A	119	618	125	$\mathrm{\underline{AB}CD}$	42.77
			B		509	163		
			C		282	211		
			D		118	39		
9-15-76	ZAPS	II	A	156	506	151	$\mathrm{\underline{AB}CD}$	26.45
			B		456	90		
			C		298	211		
			D		112	116		

---

[*] Respiration rates are expressed as μl oxygen consumed $g\text{-}hr^{-1}$, means of 3-5 samples with ±.95 confidence interval (CI).

[†] Results of one-way analysis of variance. Locations with significantly different respiration rates ($P < .05$) do not share the same underline.

APPENDIX 17.3 PERCENTAGE OF PLAMOLYSIS OF ALGAL CELLS OF *USNEA HIRTA* COLLECTED FROM ZAPS I AND ZAPS II, 1975-1976

Collection Date, Sample Location		Days SO_2	$\bar{X}$*	CI	ANOVA†	F
6-25-75 ZAPS I	A	53	6.0	9.9	$AB\underline{CD}$	17.02
	B		65.3	85.1		
	C		99.7	1.3		
	D		100.0	0		
7-10-75 ZAPS I	A	47	9.0	6.5	$AB\underline{CD}$	60.15
	B		54.3	48.6		
	C		99.0	2.6		
	D		100.0	0		
9-11-75 ZAPS I	A	110	15.3	7.7	$AB\underline{CD}$	66.39
	B		76.7	40.0		
	C		98.0	8.6		
	D		100.0	0		
5-13-76 ZAPS I	A	31	6.0	2.6	$AB\underline{CD}$	29.36
	B		34.8	39.1		
	C		94.0	21.5		
	D		94.3	22.4		
6-23-76 ZAPS I	A	72	4.0	5.2	$AB\underline{CD}$	∞
	B		77.7	3.9		
	C		99.7	1.3		
	D		100.0	0		
9-15-76 ZAPS I	A	156	7.3	3.9	$AB\underline{CD}$	58.18
	B		82.3	32.3		
	C		99.7	1.3		
	D		100.0	0		
5-13-76 ZAPS II	A	31	4.3	6.5		
	B		36.7	95.5		
	C		98.7	3.9		
	D		100.0	0		
6-23-76 ZAPS II	A	72	3.7	1.3		
	B		58.3	51.2		
	C		99.3	3.0		
	D		100.0	0		
7-17-76 ZAPS II	A	96	3.3	3.9		
	B		65.7	43.9		
	C		98.7	3.9		
	D		100.0	0		

* Means of counts of 3 samples with ±.95 confidence interval

† Results of one-way analysis of variance. Locations with significantly different plasmolysis determinations do not share the same underline.

APPENDIX 17.4 RESPIRATION RATES OF *PARMELIA CHLOROCHROA* COLLECTED FROM ZAPS I, 1975-1976

Collection Date, Sample Location		Days SO_2	$\bar{X}$*	CI	ANOVA†	F
5-1-75 Pasture‡		0	337	110		
Field‡		0	223	48		
6-26-75 ZAPS I	A	33	319	55	BACD	1.99
	B		330	45		
	C		344	26		
	D		296	42		
7-10-75 ZAPS I	A	47	345	62	BACD	5.35
	B		404	50		
	C		317	42		
	D		294	29		
9-11-75 ZAPS I	A	110	362	86	ACDB	3.56
	B		271	25		
	C		353	64		
	D		340	34		
7-14-76 Kluver East‡		0	322	65		
8-9-76 ZAPS I ground	A	23	292	120	DBCA	13.31
	B		314	62		
	C		309	65		
	D		396	60		
8-9-76 ZAPS I elevated	A	23	366	65	ABCD	39.80
	B		311	82		
	C		197	134		
	D		79	37		
9-15-76 ZAPS I ground	A	60	305	109	DCAB	7.39
	B		270	82		
	C		322	95		
	D		396	32		
9-15-76 ZAPS I elevated	A	60	345	22	ABCD	69.29
	B		174	117		
	C		45	25		
	D		43	85		

---

* Respiration rates are expressed as µl oxygen consumed g-hr^{-1}, means of 3 samples with ±.95 confidence interval (CI).

† Results of one-way analysis of variance. Locations with significantly different respiration rates (P<.05) do not share the same underline.

‡ Transplant sources.

APPENDIX 17.5 PERCENTAGE OF PLASMOLYSIS OF ALGAL CELLS OF *PARMELIA CHLOROCHROA* COLLECTED FROM ZAPS I, 1975-1976

Collection Date, Sample Location		Days SO_2	$\bar{X}$[*]	CI	ANOVA[†]	F
6-26-75 ZAPS I ground	A	33	1.7	1.7	ABCD	2.04
	B		9.0	9.9		
	C		9.8	6.7		
	D		13.3	26.1		
9-11-75 ZAPS I ground	A	110	10.3	18.9	DAC	3.17
	B		-	-		
	C		4.3	10.0		
	D		43.3	85.5		
9-15-76 ZAPS I ground	A	60	22.3	6.2	ABCD	2.62
	B		7.3	7.7		
	C		30.3	21.9		
	D		29.8	13.9		
ZAPS elevated	A	60	-	-	BCD	12.62
	B		42.7	76.5		
	C		72.0	63.6		
	D		89.8	14.6		

---

[*] Means of counts of 3 samples with ±.95 confidence interval.

[†] Results of one-way analysis of variance. Locations with significantly different plasmolysis determinations do not share the same underline.

APPENDIX 17.6. METHOD USED BY AIR QUALITY BUREAU LABORATORY, MONTANA DEPARTMENT OF HEALTH, HELENA, FOR PREPARATION AND ANALYSIS OF SULFATION PLATES

Sulfation Plates

AQ - P50 - 1 (Written 1975)

The sulfation plate for determining SO_2 concentrations is made by attaching a 4.8 centimeter diameter Gelman A fiberglass filter to a 4.8 centimeter diameter petri dish with three drops of acetone. The lead dioxide solution to coat the petri dish is made in the following manner: 112 grams of lead dioxide is blended in a blender with 700 milliliters of water, 0.7 grams of gum tragacanth, and 7 grams of fiberglass filter ground on a Wiley mill to pass 20 mesh. Ten milliliters of this material is transferred into each petri dish. The coated dish is dried in an oven at low temperature (60°C) and sealed with a lid.

To expose a sulfation plate, the lid is removed and the plate is placed in a bracket that will secure the sulfation plate upside-down. The petri dish serves as the shelter, shipping container, and lead dioxide support. The sulfation plate is exposed for approximately 30 days.

After exposure, the lead dioxide is removed from the petri dish with a little water. The insoluble lead sulfate is converted to soluble sodium sulfate with the aid of 20 ml of sodium carbonate solution (50 grams/liter) and heated in a water bath for ≃ 30 minutes. The excess insoluble lead dioxide is removed by filtration using a Whatman 42 filter. The solution is acidified with hydrochloric acid to bring the pH of the filtrate between 2 and 3. The acidified filtrate is diluted to 50 ml or any other convenient volume with water. Take a portion of this solution (up to 25 ml) and dilute to 25 ml with water. To this, 0.1 gram of sulfaver powder is added, mixed, and let stand 20 minutes. The resulting turbidity is measured in a spectrophotometer at 450 millimicrons.

The results are reported as mg $SO_3/100\ cm^2/day$.

$$\text{Sulfation rate } (\text{mg } SO_3/100\ cm^2/\text{day}) = \left[\frac{A \times 10^{-3}}{B} - C\right] 4.6 \div D.$$

A = µg SO_4 from standard curve x 10^{-3} to convert µg of SO_4 to mg of SO_4

B = Fraction of filtrate used to produce turbidity ($\frac{5}{50} = \frac{1}{10}$ if 5 ml of filtrate used)

C = Blank

D = Exposure time in days

The 4.6 conversion factor is derived as follows:

APPENDIX 17.6 (cont).

Inside diameter of sulfation plate = 4.8 cm

Radius = 2.4 cm

Area of plate = $\pi R^2 = \pi(2.4)^2 = 18.1\ cm^2$

For a 100 cm^2 would = $\frac{100\ cm^2}{18.1} = 5.52$

Then $\frac{SO_3}{SO_4} = \frac{80}{96} = 0.833$ (because you want mg SO_3)

Therefore, 5.52 x 0.833 = 4.6 (the conversion factor)

APPENDIX 17.7 LICHEN COLLECTION DATES AND DAYS OF SO_2 TREATMENT, 1975-77

	Date	Days SO_2
1975	May 24	0
	June 26	33
	July 10	47
	August 11	79
	September 11	110
1976	April 12	0
	May 13	31
	June 23	72
	July 17	96
	August 9	119
	September 14	156
1977	June 22	0
	July 6	14
	July 19	27
	August 3	42
	August 17	56
	September 14	84

SECTION 18

RESPONSES OF GROUND-DWELLING INSECTS TO SULFUR DIOXIDE

J. J. Bromenshenk

ABSTRACT

Population studies of ground-dwelling beetles and other insects were conducted using pitfall trapping at the EPA Zonal Air Pollution System (ZAPS). The studies were initiated to examine responses to in situ low level sulfur dioxide fumigation and to establish an information base for evaluation of the long-term potential for resource depletion.

The most prevalent beetles, as indicated by pitfall captures, represented three families including saprophagous, necrophagous, and predacious forms. In more than 80 percent of the trappings, more beetles were captured on the control plots than on any of the treatment plots. However, the changes in abundance as related to sulfur dioxide treatment level were most pronounced and consistent for the Scarabaeid *Canthon*, mostly *laevis*. Throughout most of the 1976 and 1977 trapping periods, the number of *Canthon* beetles captured by pitfalls was significantly lower on all of the sulfur dioxide treatment plots than on the control plots at each of the two ZAPS sites.

The decomposer beetles appear to be more important to the steady turnover of nutrients in grasslands than heretofore suspected. Observations are discussed which support this hypothesis as well as possible adverse effects of low level sulfur dioxide fumigation on other insects. Several lines of evidence are presented in support of the use of terrestrial insects as indicators of environmental quality.

INTRODUCTION

The species diversity, biomass, and abundance of insects in cool-season grasslands often surprises the initiate. More than 1,300 species of above-ground arthropods inhabit Montana rangelands; of these, approximately 1,200 are insects (Rees and Hewitt, 1977). The very abundance of insects is an indication of ecologic and economic importance.

Entomological studies have made a considerable contribution to the development of ecological concepts because insects are abundant in species and numbers, have short life spans, are small in size, easy to study, and important to ecosystems (Price, 1975).

In previous reports I have shown that specific insect systems appear to be sensitive and useful indicators or estimators of environmental quality (Bromenshenk, 1976, 1978). In these reports, I reviewed more than 200 references indicating that: (1) Air pollution has significant effects on entomological systems; (2) pollution impacts on insect systems may occur at all levels of organization from the biochemical to the ecosystem; (3) pollution may act directly on insects, as in the case of zootoxins, or indirectly, as in the case of phytotoxins that alter food and habitat resources; (4) the effects of anthropogenic contaminants on insect-plant interfaces may demonstrate reciprocity, acting on the insect through the plant or on the plant through the insect, and (5) pollution-caused changes in insect systems may induce modifications in fundamental ecosystem components and processes. Frietag *et al.* (1973), Kulman (1974), Thiele (1977), Cornaby (1977) and others reported that terrestrial arthropods, particularly predatory and saprophagous species, demonstrated considerable potential as indicators of environmental quality. Several of these studies dealt specifically with ground-dwelling beetles, although the emphasis for the most part was on the Carabids.

Pursuing the objective to identify indicators which would best serve as progenitors of pollution-induced effects on critical rangeland resources (including processes and functions of the ecosystem), I continued monitoring the population responses of ground-dwelling beetles to in situ low level SO_2 fumigation at the ZAPS sites during the 1977 growing season. Data collected in 1975 by entomologists from the Natural Resources Ecology Laboratory, Colorado State University (Dodd *et al.*, 1978), indicated that the total numbers and biomass of beetles (the Coleoptera) were substantially reduced on the high treatment plot at ZAPS I during the first year of fumigation. Since the Colorado investigators decided to concentrate their efforts on soil arthropods, I decided (Bromenshenk, 1978) to evaluate population responses of beetles to SO_2, and at the ZAPS in 1976, I observed significant decreases in the relative abundance of several species of saprophagous beetles with respect to increasing SO_2 fumigation treatment levels.

Saprophagous and necrophagous beetles breakdown and fragment organic material such as carrion, feces, and vegetative matter. They consume large quantities of these materials and distribute them through carrying and burying activities (Milne and Milne, 1976; Ritcher, 1958). Thus, they directly affect the allocation and partitioning of resources in soil/litter subsystems (Cornaby, 1975, 1977; Rainio, 1966).

I believe that saprophagous beetles have been underestimated as regards their contribution to nutrient cycling and food chains in grassland ecosysems, and in this report I shall present data in support of this hypothesis.

Obviously, beetles are not the "sole agents" of nutrient cycling. Other soil fauna, such as mites, collembolans, and millepedes, are abundant and act as comminutors of organic matter, while microbes act more directly as decomposers and as chemical deteriorators (Crossley, 1970; Dickinson and Pugh, 1974; Whitkamp, 1971). However, exclusion or depression of the abundance of litter arthropods results in lowered decomposition rates (Strojan, 1978; Whitkamp and Crossley, 1966; Payne, 1965; Edwards and Heath, 1963; Kurcheva, 1960).

In addition, litter arthropods may aid in the dispersal of soil microflora and microfauna, compete with less desirable insects such as hornflies, fleshflies, and intestinal parasites for food resources, carry mites which attack the eggs of flies and parasites, and serve as food for other insects and higher animals (MacQueen, 1975; McKinney and Morley, 1975; Price, 1975; Durie, 1975; Lodha, 1974; Bryan, 1973; Ritcher, 1958). Other benefits more directly related to their burying and comminuting activities include regulation of rates of release of nutrients, less waste of fouled herbage by grazers, better infiltration by rainfall and greater moisture holding capacity of soil, less surface runoff and loss of nutrients due to runoff, leaching, and volatilization, greater surface storage of nutrients, and better returns of phosphorus and nitrogen to the soil (McKinney and Morley, 1975; Gillard, 1967).

Although I concentrated on the effects of the SO_2 fumigation on ground-dwelling beetles, I also examined the influence of this gas on other insects, including pollinators and seed-infesting insects. Data concerning this aspect was not processed completely at the time of this report (May, 1978). I shall only briefly describe the progress here and shall include this information in the next interim report.

MATERIALS AND METHODS

In order to monitor the population responses of ground-dwelling beetles to the SO_2 fumigation treatments, I continued to use pitfall traps arranged in grid arrays across the treatment plots. Each trap consisted of a cone-shaped disposable coffee cup set into a 14-ounce disposable cold-drink cup (Olsen, Elliot, and Associates, 1976; Bromenshenk, 1978) and sunk into the soil so that the mouth was level with the soil surface. An inch of water in the bottom of each trap drowned captured beetles and prevented predacious species from devouring each other and other captured insects. All traps were baited with five grams of meat, which appeared to increase the trapping yield of Scarabaeids and Silphids and to resolve an overtrapping problem introduced when traps containing a few beetles acted as attractants.

In 1976, 24 traps were placed in a grid pattern within each treatment plot. Each trap was located 152 cm from a SO_2 delivery pipe, measured diagonally from each pipe to the ground. In 1977, I (Bromenshenk, 1978) added a trap line around the perimeter of each plot, forming a 7 x 7 Latin square grid pattern containing 25 traps within and 24 traps outside of the gas delivery lines (Figure 18.1). The perimeter lines improved our ability to monitor beetle movements. The pitfall traps were opened for intervals of ten days during the first half of each month from May through September during 1976 and during May, late June, and September of 1977.

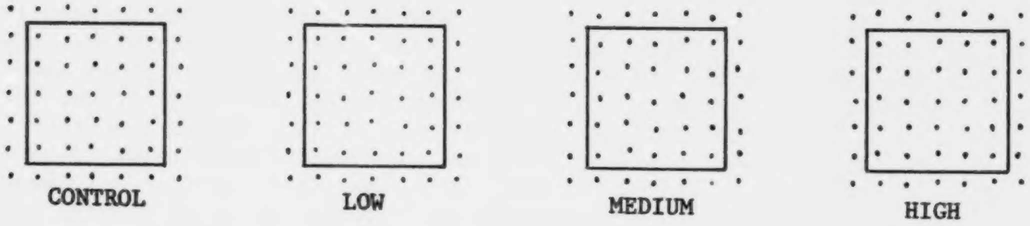


Figure 18.1. Pitfall trapping grid pattern at each ZAPS site. Boxes represent perimeters of gas delivery systems; dots represent traps.

Southwood (1975) reviewed the use of pitfall traps. He suggested that they are useful for studies of relative abundance (in similar vegetation), movements, spatial patterns of distribution, seasonal incidence, species diversity, and diurnal activity patterns (if the traps are examined frequently). However, this type of trap is difficult to use for estimates of absolute population density since animals may be attracted to the traps from a distance, and different species may vary in susceptibility to capture. Also, comparisons of captures in dissimilar vegetation types such as forest to grassland may be inaccurate because the vegetation may impede movements.

The pitfall method continues to be used because, as Kulman (1974) pointed out, other sampling methods such as mark-recapture, total population per unit area, and direct quadrant counts also create problems and are more difficult to use. Southwood (1975) concluded that the method is useful for many studies, if used with reasonable caution and if the captures are not considered to represent absolute population numbers.

Thomas and Sleeper (1977) demonstrated that the method is more suited to some species than others; as might be expected, the vagility of the population appeared to be an important factor. They listed sources of variation associated with the method, indicated methods of overcoming these problems, and reviewed mathematical models of stochastic processes. Roff (1973) disparaged the use of trapping schemes which cannot provide coefficients of variance of less than 10 percent. However, as Thomas and Sleeper (1977) explained, it is unrealistic to expect an estimate this precise for invertebrate populations; they advised acceptance of coefficients of variation as great as 100 percent.

All my statistical evaluations were performed on a Hewlett-Packard 65 calculator or a Dec-20 computer and utilized standard statistical programs, including mean, standard deviation, standard error, chi-square (goodness of fit) and linear regressions. Statistical tests and mathematical models followed the ecological methods designed for use with insect populations by Southwood (1975) and the biometrics of Sokal and Rohlf (1969) and Conover (1971).

In December, 1977, EPA released data on the SO_2 fumigation treatment levels and meteorology in the ZAPS sites area. I have incorporated mathematical evaluations testing correlations of beetle captures to SO_2 treatment and weather effects in this report and anticipate a more complete analysis for the next interim report. Also, for this report I have used only non-transformed raw data in my computations, but I intend to test for Poisson, geometric, binomial, and non-parametric distributions. If warranted, I will incorporate data transformations and appropriate statistical tests. There is a pronounced tendency towards clumping in the raw data which indicates that the populations are highly contagious. Southwood (1975) and Thomas and Sleeper (1977) demonstrated that mathematical models of distribution such as coefficients of dispersion provide important population information, and I intend to examine these processes. Currently, this trapping data is being incorporated into a Dec-20 computer program so that these more complex and lengthy statistical tests can be utilized.

The following assumptions are inherent in this study:

(1) Habitat preference is not assumed to be a major factor influencing results. Terrain and habitat features are similar on all plots at both ZAPS, although similarity does not imply identical conditions. For example, in terms of canopy cover and species diversity, the ZAPS II site is less diverse than the ZAPS I, based on 1975 and 1976 data. However, in 1976, overall vegetation similarity within sites was 0.78 on ZAPS I and 0.71 on ZAPS II (Taylor and Leininger, 1978). Other data collected at the ZAPS sites by each of the investigative teams of the CFPP project provides an extensive data base so that many confounding influences can be identified and separated.

(2) The chances that a beetle may occur on or traverse a plot or be trapped within any plot are assumed to be equal for all plots.

The null hypothesis is that relative abundance and species diversity of ground-dwelling beetles on any ZAPS plot is independent of SO_2 treatment. The corollary is that if a beetle species is sensitive to SO_2, responses to gas emitted at different concentrations across the series of treatment plots should be reflected in corresponding population changes.

Functionally, the grid trapping system should monitor resident and transient adult beetles, providing data concerning movements (dispersal, recruitment, emigration, immigration, drift) spatial patterns of distribution, seasonal incidence, species diversity, and relative abundance. These may reflect mortality factors, which were not differentiated from behavioral responses in this study.

Several terms and names used throughout this paper are rather specialized and require a brief explanation:

(1) Perimeter trap--any trap outside of the area of a treatment plot enclosed by the SO_2 gas delivery lines; in other words, any trap in the outermost rows and columns of the 7 x 7 Latin Square grid.

(2) Interior trap--any trap within the area of a treatment plot enclosed by the SO_2 gas delivery lines; any trap in the central (5 x 5) portion of the 7 x 7 Latin Square grid.

(3) *Canthon*--beetles, mostly of the species *C. laevis* (Drury), which are members of the subfamily Coprinae and which feed upon decaying substances such as carrion, vegetative matter, and dung (Ritcher, 1958). Since some species of *Canthon* can be segregated only by characteristics of the male genetalia, a process which would necessitate extremely time-consuming dissections, species have not been differentiated in the counts. However, samples which I did dissect indicated that 95 percent of the *Canthon* beetles were of the species *laevis*.

(4) Saprophagous--feeding on decaying organic matter.

(5) Coprophagous--feeding on dung.

(6) Necrophagous--feeding on carrion or dead bodies.

(7) Nidification--nest building or provision for the progeny by adults such as the dung balls rolled by *Canthon*.

(8) Scarabaeidae--the family of lamellicorn beetles composed mainly of stout-bodied, saprophagous or phytophagous beetles.

(9) Silphidae--the family of clavicorn beetles comprised of the burying beetles, carrion beetles, and related forms.

RESULTS

Pitfall trapping results for the 1976 season were published previously (Bromenshenk, 1978; Bromenshenk and Gordon, 1978) and will only be summarized here. Trapping results for the 1977 season have been completed for all three trapping periods. In May, 1976, I observed significant decreases on the treatment plots in the numbers of five species of beetles representing three families: Scarabaeidae, Silphidae, and Carabidae. The decreases were negatively correlated with fumigation levels. Depressions in abundance were most pronounced and consistent through the season for *Canthon laevis*. In general, the population levels of the other species declined and remained low across the plots (including control) throughout the summer and autumn. Captures of *Canthon* sp. in August, 1977, displayed the same pattern as early season captures (Figure 18.2).

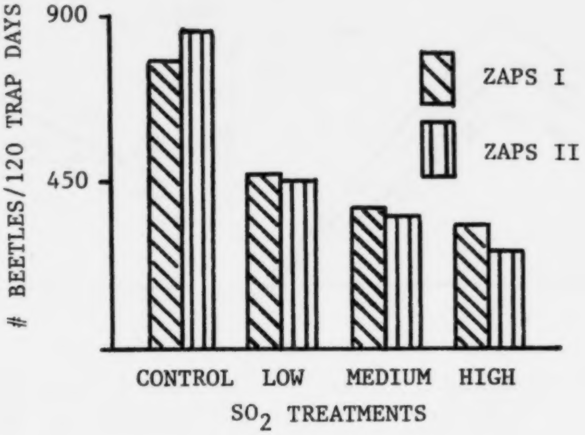


Figure 18.2. Total captures of *Canthon* sp. in August, 1976, at the ZAPS.

Results from 1977 (Figures 18.3 and 18.4) continued to demonstrate for *Canthon* beetles a decrease in abundance as SO_2 treatment increased. There was a time-lag before this effect was seen. During the May trapping period, there was little difference between the numbers of *Canthon* on the control plot (A) and the high fumigation plot (D) on ZAPS I, although there were fewer of *Canthon* captured on D of ZAPS II than on A. The ZAPS gas delivery systems had been in operation 35 days by the end of the May trapping period. By mid-summer, at both ZAPS I and II, population changes were very pronounced and continued through the September trapping period. The total population size of *Canthon*, as indicated by captured beetles, was about the same at ZAPS I and II, and the populations appeared to peak in mid-summer and decline by September.

There was a pronounced edge capture effect indicating movement onto, off, or across the plots. It should also be noted that the changes in population abundance of *Canthon* relative to SO_2 treatment level were most pronounced on the interiors of the plots (Figure 18.4). The population abundance of *Canthon* on the control (A) plots was, for the most part, significantly greater (χ^2) than for any of the treatment plots, except during May. Differences in abundance between the low (B), medium (C), and high (D) treatment levels were variable, sometimes significant (χ^2) for one and sometimes for the other.

Summaries of capture data for all species and the basic statistics performed appear in Appendix Tables 18.1 through 18.9. Changes in the relative abundance of other species of beetles captured at the ZAPS sites in 1977 as related to SO_2 treatment levels were not as easily interpretable as those of *Canthon*. Again, perimeter traps tended to capture more insects than

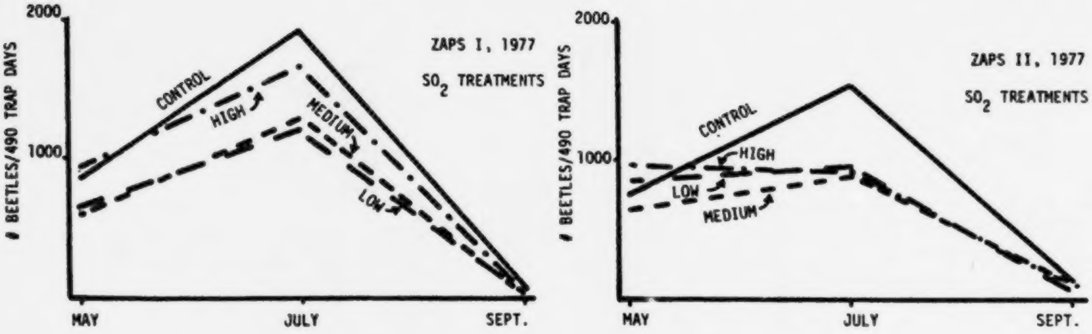


Figure 18.3. Seasonal summary of captures of *Canthon* sp. beetles by traps both interior and exterior to the plots.

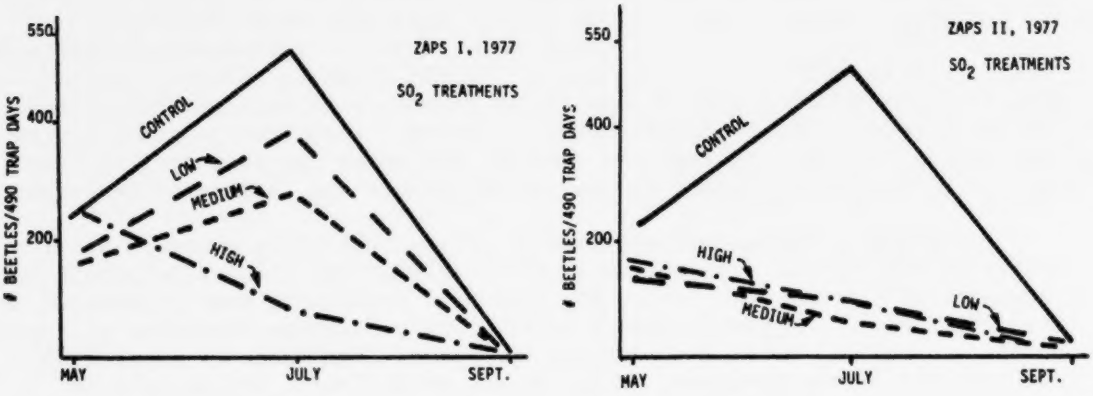


Figure 18.4. Seasonal summary of captures of *Canthon* sp. beetles by traps on the interior of the plots.

interior traps. Unlike *Canthon*, not all of these other groups of beetles reached maximum population size by mid-summer. Scarabaeids of the species *Onthophagus hecate* Panz. (small, saprophagous and necrophagous beetles) were captured most frequently in May as were weevils of the family Curculionidae. Skin, feather, and dung-feeding beetles of the genus *Trox*, subfamily Troginae, were most common in September. Carrion feeders of the genus *Nicrophorus* (Silphidae) were caught at about the same rate throughout the growing season. Carabids of the predacious species *Pasimachus elongatus* LeConte were most abundant during May and June-July, while predatory tiger beetles of the genus *Cicindela* (Cicindelidae) often were caught in May and September, but none were captured in June-July. Miscellaneous species of beetles which were captured infrequently were grouped in a category designated "other beetles." They were most numerous in May, common in June-July, and greatly reduced in number by September.

Because of the in-line placement of the ZAPS treatment plots, the B and C plots bordered two 61-meter intervening buffer zones, while the A and D plots bordered only one buffer zone. The north and south portion of the plots interfaced with the surrounding rangeland. More beetles should have inhabited the surrounding grasslands, which extended for kilometers in all directions, than inhabited the 61 x 35 m buffer zones. If all species of beetles had very limited home ranges, this would not be important. But some of the decomposer beetles have extensive home ranges. For example, Milne and Milne (1976) described experiments by F. Petruska in which carrion beetles that were carried as far as four kilometers from a carcass returned within 24 hours. But, Shubeck (1968) concluded that *Nicrophorus* sp. seemed to utilize random wandering to carrion and were not very efficient in locating carrion even from distances as close as five meters, although he postulated that there may be species and even population differences in patterns of foraging behavior. My own field observations indicated that although both *Canthon* and *Nicrophorus* beetles often were seen moving about on the ground, they are strong fliers. I have seen them traverse the length of a treatment plot quickly, sometimes traveling back and forth across one or two plots several times before disappearing from my visual range.

If the relative abundance and species diversity of ground-dwelling beetles were not affected by SO_2 treatment, then one would expect an equal probability of capture on each of the plots for those beetles that have a limited home range, that is, areas smaller than the buffer zones. However, for beetles that can and do move distances greater than the width of the buffer zones during the ten-day trapping interval, an equal probability of capture was hypothesized for plots bordered on three sides by rangeland (A and D plots) and for plots bordered on two sides by rangeland (B and C plots) with a greater rate of capture on the former compared to the latter. This phenomenon is termed edge effect. As an alternative scenario, it was hypothesized that capture rates would not be equal on each of the plots because of SO_2 treatment effects and that the plots receiving the least amount of SO_2 would display the highest capture success, since the 1976 trapping data demonstrated inverse correlations between beetles and SO_2 levels.

These hypotheses were tested by pairing the trapping data for all generic groups captured on at least some of the plots during all three trapping periods. Then the non-parametric signs test was applied. This is based on the hypothesis that the number of positive and negative signs among differences (omitting all differences of zero) occurred in equal frequencies.

For limited beetle movement and home range, the following hypotheses were tested:

(1) $H_0 : P(A < B) \geq 1/2$

$H_1 : P(A < B) < 1/2$

(2) $H_0 : P(B < C) \geq 1/2$

$H_1 : P(B < C) < 1/2$

(3) $H_0 : P(C < D) \geq 1/2$

$H_1 : P(C < D) < 1/2$

But if beetle movements and home range were large, edge effects would be important and only homologous pairs could be tested, i.e., AD or BC. The hypotheses applicable in this instance were:

(1) $H_0 : P(A < D) \geq 1/2$

$H_1 : P(A < D) < 1/2$

(2) $H_0 : P(B < C) \geq 1/2$

$H_1 : P(B < C) < 1/2$

The results of the sign tests are presented in Table 18.1.

Based on the sign test analysis for perimeter traps, I accepted the hypothesis of an edge effect; i.e., $A > B$ ($P = .09$) and $D > C$ ($P = .03$). For the interior traps, I rejected the hypotheses of equal captures. The data indicated that capture success followed the trend $A > B > C = D$, the differences significant at the $P < .001$ level for $A > B$, at the $P < .10$ level for $B > C$, but not significant for $C > D$. This supports the hypothesis of a treatment effect which demonstrated, after an initial effect, a pattern of diminishing effects with increasing SO_2. Other statistical tests will be applied, but these were not complete at the time of this draft.

TABLE 18.1 SIGN TEST OF BEETLE CAPTURES FOR THE FIVE MOST PREVALENT GENERA AND FOR ALL TRAPPING PERIODS, ZAPS I AND II, 1977

Interior Traps	Perimeter Traps	Incidence of Property Number	Incidence of Property Percentage	Exact Probability (one-tailed test)
A > B		24	85.7	.9999
A < B		4	14.3	.0001
B > C		18	66.7	.939
B < C		9	33.3	.061
C > D		13	56.5	.661
C < D		10	43.5	.339
A > D		23	85.0	.998
A < D		4	15.0	.0002
	A > B	18	64.3	.908
	A < B	10	35.7	.092
	B > C	15	46.4	.425
	B < C	13	53.6	.575
	C > D	8	70.4	.974
	C < D	19	29.6	.026
	A > D	13	44.8	.355
	A < D	16	55.2	.645

Sulfation plate studies (Section 10) indicated that in the buffer zones, sulfur fumigation levels at 35 cm above the ground averaged 53 percent of the nearest sample points inside the treatment plots. The perimeter beetle traps and the buffer zone sulfation plates were located at approximately the same distance from the edge of the plots. Decreased sulfur fumigation at the perimeters of the plots, as compared to the interiors, could account for the difference in the capture rate between perimeter traps and interior traps.

In my data analyses of beetle capture relative to SO_2 treatment level, I used the EPA sulfation plate data (Section 10). This provided the largest amount of information on horizontal and vertical spatial distributions. Unless otherwise indicated, only data from the 35 cm height was used, the lowest height for which information was available for all trapping periods. It was assumed that ground level concentrations would have the greatest effects on the responses of ground-dwelling beetles, although this may not be true; long range movements appeared to be mainly by flying.

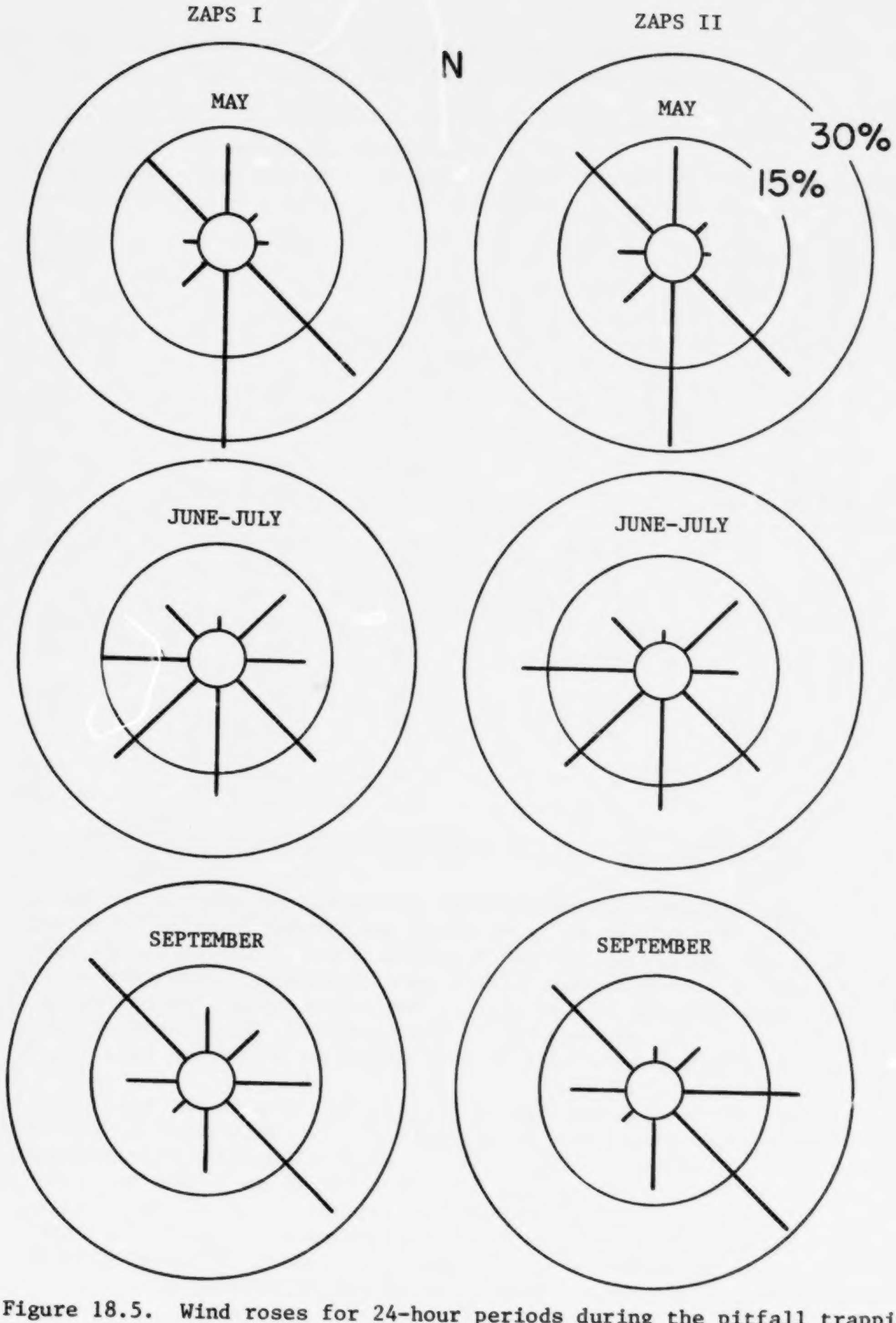


Figure 18.5. Wind roses for 24-hour periods during the pitfall trapping of beetles at the ZAPS; length of line indicates frequency.

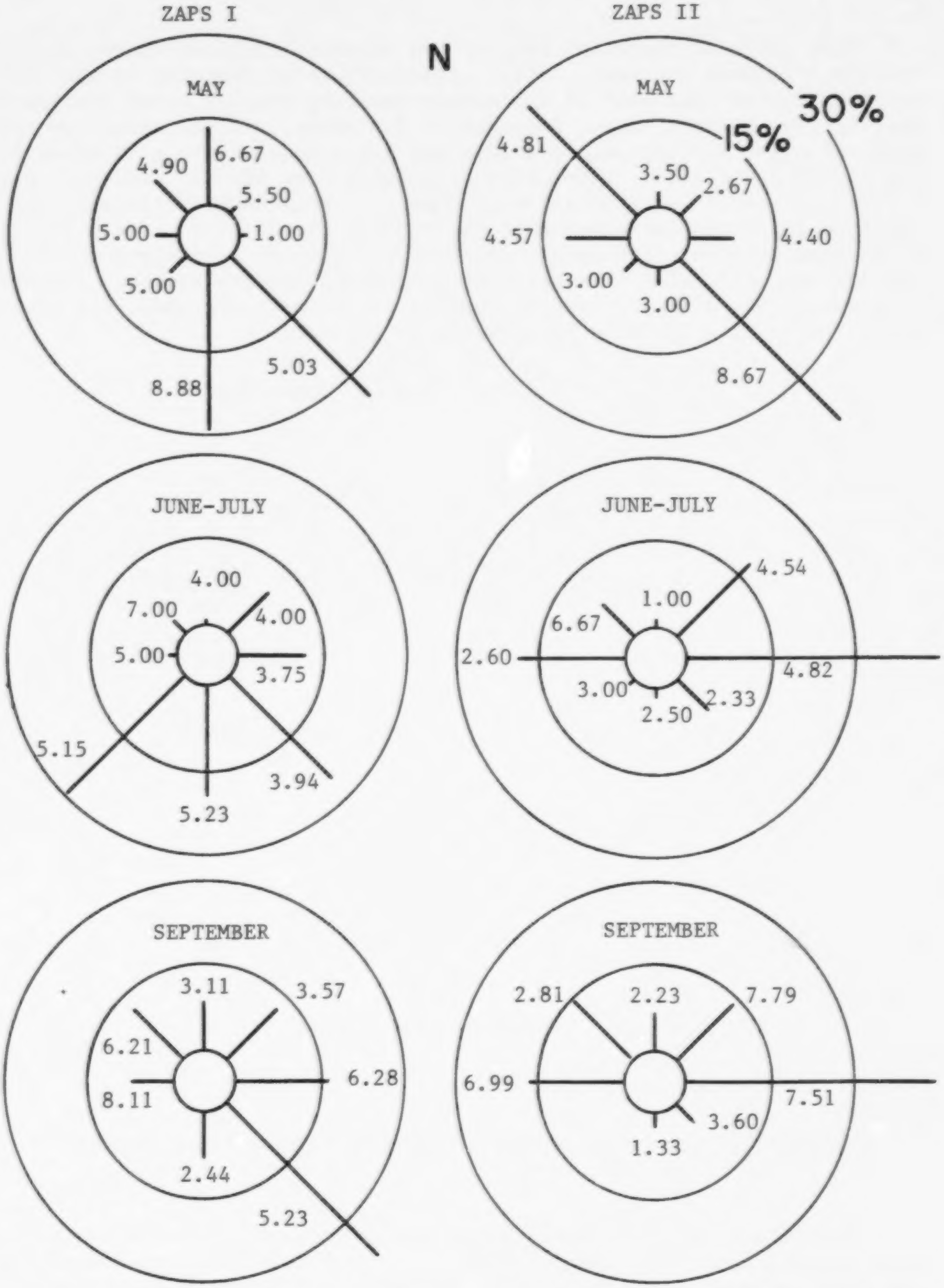


Figure 18.6. Wind roses for night periods during the pitfall trapping of beetles at the ZAPS; numbers indicate wind speed and length of line indicates frequency.

Wind plays an important role in the orientation of movements by many insects (Fraenkel and Gunn, 1961). Observations of captures by the pitfall traps indicated that many of the ground-dwelling beetles being studied were most active at night, i.e., nocturnal. Therefore, wind patterns for the 24-hour and night periods were examined and are summarized by wind roses in Figures 18.5 and 18.6. Winds blew frequently from the SO_2 treatment plots towards the controls, especially at night, at both ZAPS. This supports data (Section 10) which indicated that SO_2 reached the control plots. Comparison of Figures 18.5 and 18.6 (wind roses) with Figure 18.7 (perimeter captures of *Canthon* sp.) indicates that there is a tendency for the greatest capture rates to occur on the side of the plots upwind of the more prevalent air flows. These trends have not been mathematically analyzed.

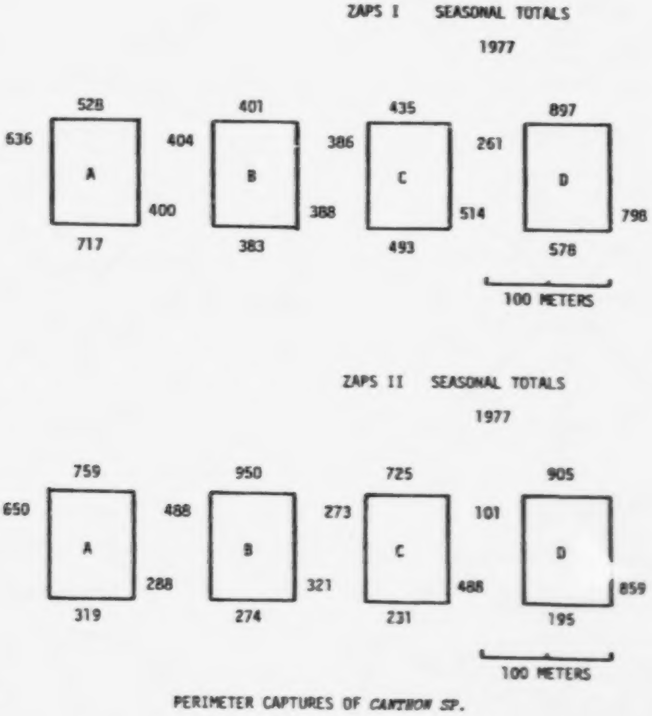

ZAPS I SEASONAL TOTALS
1977
528
401
435
897
636
A
404
B
386
C
261
D
400
388
514
798
717
383
493
578
100 METERS
ZAPS II SEASONAL TOTALS
1977
759
950
725
905
650
A
488
B
273
C
101
D
288
321
488
859
319
274
231
195
100 METERS
PERIMETER CAPTURES OF *CANTHON SP.*

Figure 18.7. Seasonal totals of perimeter captures of *Canthon* sp. at ZAPS.

Statistical analyses of wind and sulfur interactions with beetle captures were in progress at the time of this report. Linear regression analysis of the seasonal capture of *Canthon* sp. against the reciprocal of the sulfation concentrations was performed. The reciprocal transformation produced a significant r^2 value (F = 23.847, df = 1,6). The captures of *Canthon* sp. were inversely correlated to sulfate concentration (Figure 18.8).

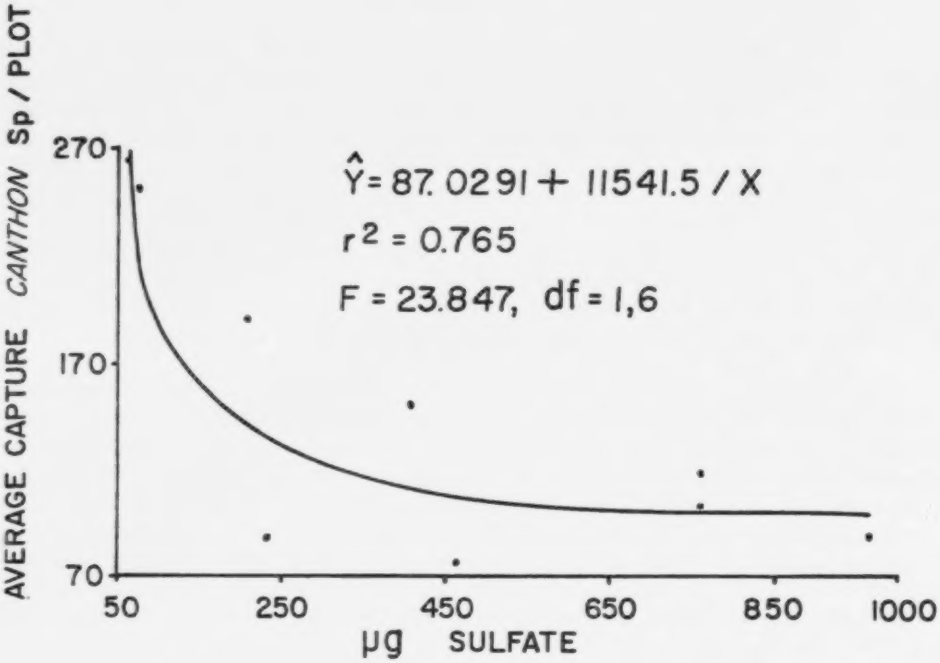


Figure 18.8. Linear regression of seasonal capture of *Canthon* sp. against the reciprocal of sulfation concentrations.

I continued to maintain hives of honeybees at ZAPS II in 1978. One hive was located north of each of the plots 35 meters from the last delivery pipe. It was hoped that a line could be extended to the hives to insure SO_2 fumigation, but this was not done. The hives were not located on the plots because of concern expressed by other investigators about the possibility of being stung by bees while working on the plots. As in previous years, bees continued to abscond from hives above the higher treatment plots. This may be coincidental, but Hillmann (1972) had a similar problem when he fumigated bees with SO_2 at levels up to 5 ppm.

Seed germination of *Koeleria cristata* (Section 14) revealed that thrips frequently infest seeds but that the percent infestation changes across the treatment plots. At ZAPS I in 1977, thrips were found in 5, 3, 3, and 1 percent of 100 seeds examined from A to D, respectively.

Another SO_2 effect on insect feeding was a preference for unfumigated versus fumigated grass by grasshoppers (Section 19).

Thus, insects at the ZAPS during 1977 demonstrated significant and varied responses to the SO_2 treatments.

DISCUSSION

Based on the trends of preliminary statistical analyses, population abundance relative to SO_2 treatments clearly demonstrated that chronic low levels of SO_2 affected *Canthon* beetle populations. The data indicated that more of these beetles were captured within the control plots than on any of the fumigated plots. Either beetles moved farther into the interior of the control plots or there was a larger resident population in the interior of these plots. It appeared that SO_2 often caused a significant reduction in abundance even at the lowest (B) fumigation levels and that increased fumigation (C and D plots) demonstrated only a slightly greater reduction in abundance. This suggests that whatever the threshold for this response might be, it occurs at or below the lowest fumigation concentration.

Trapping success of *Canthon* greatly increased by mid-summer and decreased in the autumn. Both seasonal changes of abundance and treatment related changes in abundance were more or less parallel at ZAPS I and ZAPS II, although the responses were more consistent and displayed a steeper gradient of decrease on ZAPS II. Data on sulfur uptake by vegetation (Section 14) showed a steeper gradient of sulfur uptake on ZAPS II, while capture-mark-release studies of deer mice (*Peromyscus maniculatus*) carried out by Chilgren (1978 and Section 20) yielded results which were very similar to those of my beetle trapping. Based on trapping data from 1977 and 1976, the number of mice that he captured on all fumigated plots decreased relative to the control plots on both ZAPS. The effect became most pronounced at mid-season, continued through the autumn, and was more clearly demonstrated on ZAPS II than on ZAPS I.

Chilgren pointed out that the two most important findings of his work were that: (1) Sampling must be carried out over extended periods of time before effects become interpretable and (2) similar results occurred when the same experiment was replicated in space (ZAPS I and II) and time (1976, 1977, and within season). I concur with his interpretation of his results, and these statements also apply to my data.

Canthon laevis may be more sensitive or susceptible to SO_2 pollution than other grassland, ground-dwelling beetles, but other beetles, including saprophagic and predatory forms, have been shown to respond in a similar manner to sulfur compounds in the ambient air (Bromenshenk, 1978; Dodd *et al.*, 1978; Frietag *et al.*, 1973). The sensitivity of other species of insects and their usefulness in studies of sulfur oxide pollution have not been determined. Preliminary data have shown a trend towards decreased seed infestation of prairie Junegrass by thrips (Section 14) and significant preferences by a grasshopper, *Aulocara elliotti* (Section 19), for non-fumigated western wheatgrass over that from the highest fumigation plot. Hillmann (1972) reported increases of aphids and decreases of predatory and parasitic wasps (Hymenoptera) near a 615 MW coal-fired power plant in Pennsylvania.

Most previous studies of the impacts of pollutants on terrestrial insects systems have consisted of the formulation of species lists and other types of unfocused but extensive information or the collection of data which is by nature so variable as to be unusable in evaluating chronic effects. It is the functioning of components and processes of ecosystems which is of interest to ecologists, policy makers, and managers, and this functioning needs to be understood in order to provide the information necessary for environmental problem solving. Because of this, I believe that studies of pollution impacts on terrestrial insects should concentrate on critical (key) or sensitive insect systems rather than on broad spectrum sampling. In addition, the ability to delineate effects is dependent to a great degree on the utilization of appropriate methods of investigation. For example, D-Vac sampling of aboveground arthropods provided some leads towards identifying sensitive species, but the variability of the data made the investigators hesitant to attribute any observed changes to treatment effects (Dodd *et al.*, 1978). D-Vac techniques are poorly suited to examinations of many ground-dwelling beetles which tend to hide under objects during the day and to be most active at night, while pitfalls are effective both day and night.

At this point, some difficult questions have been raised. Given the large number of insect species present in grassland ecosystems, do other important insects (other than the ones identified) respond to SO_2? Has the inability to identify other susceptible insects been due to inadequate approaches, lack of concentration on the specific group, or insufficient time for a response to become evident? In view of the bioaccumulation of fluorides and arsenic near power plants and smelters and the toxic effects of these substances on bees (Bromenshenk, 1978; Section 6), what are the effects of other pollutants such as cadmium, mercury, polycyclic hydrocarbons, fluorides, and arsenics, alone or in synergism with sulfur oxides, on insect systems? The ZAPS studies have provided a means of finding sensitive, preliminary indicators. Now there is a need to know what changes in fundamental ecosystem components and processes should be anticipated as a consequence of responses by insect systems.

The depressed abundance of *Canthon* beetles on the fumigation plots may reflect some type of disorientation or avoidance movement, mortality of a resident population, or some combination of mortality and behavioral factors. The mechanisms involved will be the focus of my 1978 investigations.

A hypothesis has been advanced that if the reduced abundances observed during this study were a result of alterations in movements or distributional habitat choices, there would be no changes in the abundance of these beetle populations in SO_2-polluted areas. The rationale for this hypothesis is that the beetles in this study were avoiding a very localized SO_2 fumigation but would not have the opportunity to do so if large areas were involved. Also, if extensive geographical areas were inundated by SO_2, the question arises as to whether insects could adapt (or develop resistant populations, if mortality is a factor) to SO_2? If this occurred, there may be no long-term effects on the grassland ecosystem once the system essentially adjusts itself to the prevailing conditions.

On the other hand, avoidance or distributional habitat choices may convey protection from toxic effects by SO_2, while disorientation could impair the efficiency and success of finding of food resources. Movements could proceed from areas of higher concentration of SO_2 to lower. Thus, even if beetles were not capable of "escaping" SO_2 stress by an extended emigration, this might occur over several generations or over long periods of time by a series of shorter incremental movements. Small rodent studies by Chilgren (Section 20) indicated that mice may avoid SO_2. Because carrion is an important food resource to many of these beetles, they might follow small rodent movements.

If resistance were to become a factor in the response of these beetles to SO_2 stress, one would expect that SO_2 dosages would have to be high enough to kill a segment of the population. Stress avoidance could negate this possibility. Also, Brown (1968) pointed out that there is no "single doctrinaeire answer" to resistance phenomena.

Frietag *et al.* (1973) found depressed abundance of several species of ground-dwelling beetles in the plume path of a Kraft mill, and Hillmann (1972) observed reductions of predatory Hymenoptera downwind from a coal-fired power plant complex 14 years after the plant began operations. These indicate that reductions in insect population abundance have occurred and may persist for several years, although different mechanisms of action may be involved. Regardless of the mechanisms involved, a continued depression of the abundance of these beetles could have long-term effects on grassland ecosystems.

The trophic relations of coprophagous and necrophagous beetles have been described (Milne and Milne, 1976; Ritcher, 1958; Price, 1975), and personnel of the Environmental Studies Laboratory are obtaining data on the relationship of these beetles to mice and voles, both as food to these rodents and as consumers of them. This should provide data concerning food web effects.

I am particularly interested in the role that these insects play in soil nutrient cycles. Necrophagous and coprophagous beetles appear to occur in greater numbers in eastern Montana grasslands than in western Montana forests, based on comparisons of our data with that of the USDA Region I Forest Service which is trapping beetles in pitfalls in timber stands (personal communication, D. Fellin, USDA Entomologist). If this finding is typical, studies of soil nutrient cycles in forest systems would not consider these beetles to be of much importance due to low population numbers. However, in the grasslands, as many as 1,000 beetles of a single species have been captured on a 1½-acre plot over a ten-day period. In addition, 20 percent of the mice caught in snap traps near Colstrip by our personnel during the 1977 growing season were attacked, partially devoured, and often buried by carrion beetles during the same night that they were caught. F. Munshower (MSU) and J. Chilgren (EPA) (personal communications) reported that mice, voles, and ground squirrels in their traps were badly mutilated and sometimes the smaller animals completely stripped of flesh by beetles within as short a period as 24 hours. These were animals that had been inadvertently captured or overlooked so that they had not been more quickly removed from the traps. Milne and Milne (1976) reported that adult burying beetles transport and bury bodies of birds and small animals as large as 100 gm, or the size of a rat or large robin, and that they may inter larger animals, birds, or snakes. They credit these beetles with the removal of the majority of dead carcasses of small animals and birds. Thus, it appears that decomposer beetles (saprophages and necrophages) are important to the rapid turnover of the organic materials in grassland ecosystems.

CONCLUSIONS

As in any field study, innumerable variables could not be controlled, although the employment of support data such as meteorology, vegetation maps, SO_2 fumigation, and uptake (vegetation, sulfation plates) in the data analyses should greatly improve the ability to partition sources of variance.

However, based on the capture of 17,159 *Canthon* sp. beetles at ZAPS I and II in 1977, during these trapping periods (May thru September), the data strongly indicate that chronic low levels of SO_2 affect highly mobile populations of these beetles. I am currently examining sulfur accumulation in the body tissues of beetles, but these analyses were not available for incorporation into this report. Thus, at present, I have no physiological data on SO_2-fumigated beetles and no data concerning life table factors such as age structure, natality, mortality, life expectancy, and reproductive processes.

Analysis of temporal and spatial capture patterns of *Canthon* sp. indicated that:

(1) Total captures, with the exception of the May trapping period on ZAPS I, were significantly greater (χ^2 analyses) on the controls than for any of the fumigated plots.

(2) Population size, as reflected by total capture, was greatest in mid-summer and smallest by mid-September.

(3) The effects of SO_2 fumigation demonstrated a noticeable time-lag as reflected by beetle capture; *i.e.*, early season trapping for the most part showed no or little SO_2 effect on the population abundance.

(4) Temporal and treatment-related changes were more or less parallel on ZAPS I and II, although decreased relative abundance as compared to increased SO_2 treatment on ZAPS II tended to be more consistent and pronounced.

(5) Perimeter capture exceeded interior captures in all cases, indicating recruitment or movement onto the plots. Assuming that perimeter traps should be the first to be occupied by immigrants and nearby residents and should contain a larger portion of the capture, one would expect that the interior traps would fill at equal rates with residents and those beetles which successfully eluded "the filter effect" of the perimeter traps, providing SO_2 does not affect the populations.

(6) Capture rates within plots demonstrated higher levels of difference in beetle abundance across the treatment plots than total captures, especially for the controls. Either there is a larger resident population or movements are less restricted at the control plots.

(7) Although demonstrating a trend towards an inverse correlation of numbers compared to fumigation level, changes in abundance within the fumigated plots were variable.

(8) The data indicate that SO_2, even at the lowest fumigation level, affected free ranging populations of *Canthon*.

(9) The results for 1977 at the ZAPS are consistent with the results obtained in 1976.

Thus, although I do not know how a beetle such as *Canthon* responds to SO_2, the results of these tests indicate that SO_2 significantly affects the relative abundance of beetles in grasslands, causing depressed abundance. Generic differences in sensitivity of ground-dwelling beetles to SO_2 air pollution may occur, although results from 1976 (Bromenshenk, 1978) indicate that several different species and families respond to SO_2.

Saprophagous, including coprophagous and necrophagous, beetles as well as predatory beetles contribute to the vigor of grassland ecosystems. Damage to decomposers or nutrient pools may constitute a potential source of instability to the entire ecosystem (Harte and Levy, 1975; Dudzik *et al.*, 1975). Cornaby (1977) presented three resource problems of international importance to which saprophagous animals may provide a key to the solution and an understanding of ecosystem processes. He concluded that saprophagous animals do or can contribute to: (1) Assessments of environmental quality (biological indicators); (2) acceleration of the regeneration of spent or reclaimed land, and (3) deactivation of pathological materials.

The long-term consequences of reduced abundance of saprophagous and predatory beetles are unclear, but a potential of important changes in ecosystem functioning seems likely.

REFERENCES

Bromenshenk, J.J. 1976. Investigations of the Effects of Coal-Fired Power Plant Emissions Upon Insects, Report of Progress. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Second Interim Report, Colstrip, Montana, June, 1975, R.A. Lewis, N.R. Glass, and A.S. Lefohn, eds. EPA-600/3-76-013, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 112-129 and 286-312.

________________. 1978. Investigations of the Impact of Coal-Fired Power Plant Emissions Upon Insects. I. Entomological Studies in the Vicinity of Colstrip, Montana. II. Entomological Studies at the Zonal Air Pollution System. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Third Interim Report, Colstrip, Montana, December, 1977, E.M. Preston and R.A. Lewis, eds. EPA-600/3-78-021, U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 146-312 and 473-507.

________________, and C.C. Gordon. 1978. Terrestrial Insects Sense Air Pollutants. In: Proceedings of the Fourth Joint Conference on Sensing of Environmental Pollutants, November 6-11, 1977, New Orleans, Louisiana. American Chemical Society, Washington, D.C. pp. 66-70.

Brown, A.W.A. 1968. Insecticide Resistance Comes of Age. Bull. Entomol. Soc. Am., 14:3-9.

Bryan, R.P. 1973. The Effects of Dung Beetle Activity on the Numbers of Gastro-Intestinal Helminth Larvae Recovered from Pasture Samples. Aust. J. Agric. Res., 24(1):161-168.

Chilgren, J.D. 1978. Responses of Prairie Deer Mice to a Field SO_2 Gradient. In: Proceedings of the Fourth Joint Conference on Sensing of Environmental Pollutants, November 6-11, 1977, New Orleans, Louisiana. American Chemical Society, Washington, D.C. pp. 61-65.

Conover, W.J. 1971. Practical Nonparametric Statistics. John Wiley & Sons, Inc., New York. 462 pp.

Cornaby, B.W. 1975. Soil Arthropods as Indicators of Environmental Quality. In: Organisms and Biological Communities as Indicators of Environmental Quality--A Symposium, C.C. King and L.E. Elfner, eds. The Ohio State University, Columbus, Ohio. pp. 23-25.

________. 1977. Saprophagous Organisms and Problems in Applied Resource Partitioning. In: The Role of Arthropods in Forest Ecosystems, W.V. Mattson, ed. Springer-Verlag, New York. pp. 96-101.

Crossley, D.A., Jr. 1970. Roles of Microflora and Fauna in Soil Systems. In: Pesticides in the Soil: Ecology, Degradation, and Movement. International Symposium on Pesticides in the Soil. Michigan State University, East Lansing, Michigan. pp. 30-35.

Dickinson, C.H., and G.J.F. Pugh, eds. 1974. Biology of Plant Litter Decomposition, Volumes 1 and 2. Academic Press, New York. 146 pp. and 175 pp.

Dodd, J.L., W.K. Laurenroth, R.K. Heitschmidt, and J.W. Leetham. 1978. First-Year Effects of Controlled Sulfur Dioxide Fumigation on a Mixed Grass Prairie Ecosystem. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Third Interim Report, Colstrip, Montana, December, 1977, E.M. Preston and R.A. Lewis, eds. EPA-600/3-78-021. U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 345-375.

Dudzik, M., J. Harte, D. Levy, and J. Sandusky. 1975. Stability Indicators for Nutrient Cycles in Ecosystems. Lawrence Berkeley Laboratory Report LBL-3264. University of California, Berkeley, California. 59 pp.

Durie, P. 1975. Some Possible Effects of Dung Beetle Activity on the Infestations of Pastures by Intestinal Worm Larvae of Cattle. J. Appl. Ecol., 12(3):827-831.

Edwards, C.A., and G.W. Heath. 1963. The Role of Soil Animals in Breakdown of Leaf Material. In: Soil Organisms, J. Doeksen and J. van der Drift, eds. North-Holland Publishing Company, Amsterdam. pp. 76-84.

Fraenkel, G., and D.L. Gunn. 1961. The Orientation of Animals, Kineses, Taxes, and Compass Reactions. Dover Publications, Inc., New York. 376 pp.

Frietag, K., L. Hastings, W.R. Mercer, and A. Smith. 1973. Ground Beetle Populations Near a Kraft Mill. Can. Entomol., 105(2):299-310.

Gillard, P. 1967. Coprophagous Beetles in Pasture Ecosystems. J. Aust. Inst. Agric. Sci., 33(1):30-34.

Harte, J., and D. Levy. 1975. On the Vulnerability of Ecosystems Disturbed by Man. In: Unifying Concepts in Ecology, W.H. van Dobben and R.H. Lowe-McConnell, eds. Junk Publications, The Hague, Netherlands. pp. 208-223.

Hillmann, R.C. 1972. Biological Effects of Air Pollution on Insects, Emphasizing the Reactions of the Honey Bee (*Apis mellifera* L.) to Sulfur Dioxide. Ph.D. Thesis, The Pennsylvania State University, University Park, Pennsylvania. 159 pp.

Kulman, H.M. 1974. Comparative Ecology of North American Carabidae with Special Reference to Biological Control. Entomophaga Mem. H.S., 7:61-70.

Kurcheva, G.F. 1960. Role of Invertebrates in the Decomposition of Oak Leaf Litter. Povcvovedenie, 4:16-23.

Lodha, B.C. 1974. Decomposition of Digested Litter. In: Biology of Plant Litter Decomposition, Volume 1, C.H. Dickinson and G.F. Pugh, eds. Academic Press, New York. pp. 213-238.

MacQueen, A. 1975. Dung as an Insect Food Source: Dung Beetles as Competitors of Other Coprophagous Fauna and as Targets for Predators. J. Appl. Ecol., 12(3):821-827.

McKinney, G.T., and F.H.W. Morley. 1975. The Agronomic Role of Introduced Dung Beetles in Grazing Systems. J. Appl. Ecol., 12(3):831-837.

Milne, L.J., and M. Milne. 1976. The Social Behavior of Burying Beetles. Sci. Am., 235(2):84-89.

Olson, Elliott, and Associates. 1976. Effects of Spruce Budworm Control on Pollinating Insects. USDA Forest Service, Region 1, Missoula, Montana. 56 pp.

Payne, J.A. 1965. A Summer Carrion Study of the Baby Pig *Sus scrofa* Linnaeus. Ecology, 46(5):592-602.

Price, P.W. 1975. Insect Ecology. Wiley-Interscience, New York. 514 pp.

Rainio, M. 1966. Abundance and Phenology of Some Coprophagus Beetles in Different Kinds of Dung. Ann. Zool. Fennici., 3(1):88-98.

Rees, N.E., and G.B. Hewitt. 1977. Effects of Specific Cultural Practices on Immediate Rangeland Arthropod Populations. Bulletin 695. Montana Agricultural Experiment Station, Montana State University, Bozeman, Montana. 111 pp.

Ritcher, P.O. 1958. Biology of Scarabaeidae. Ann. Rev. Entomol., 3:311-335.

Roft, D.A. 1973. On the Accuracy of Some Mark-Recapture Estimators. Oecologia (Berl.), 12(1):15-34.

Shubeck, P.P. 1968. Orientation of Carrion Beetles to Carrion: Random or Non-random? J. N.Y. Ent. Soc., 76(4):253-265.

Sokal, R.R., and F.J. Rohlf. 1969. Biometry. The Principles and Practice of Statistics in Biological Research. W.H. Freeman & Company, San Francisco, California. 776 pp.

Southwood, T.R.E. 1975. Ecological Methods with Particular Reference to the Study of Insect Populations. Chapman and Hall Ltd., London. 391 pp.

Strojan, C.L. 1978. The Impact of Zinc Smelter Emissions on Forest Litter Arthropods. Oikois (in press).

Taylor, J.E., and W.C. Leininger. 1978. Monitoring Plant Community Changes Due to SO_2 Exposure. In: The Bioenvironmental Impact of a Coal-Fired Power Plant, Third Interim Report, Colstrip, Montana, December, 1977, E.M. Preston and R.A. Lewis, eds. EPA-600/3-78-021. U.S. Environmental Protection Agency, Corvallis, Oregon. pp. 376-384.

Thiele, H.-U. 1977. Carabid Beetles in Their Environments. A Study in the Habitat Selection by Adaptations in Physiology and Behavior. Springer-Verlag, Berlin, Fed. Rep. Germany. 369 pp.

Thomas, D.B., Jr., and E.L. Sleeper. 1977. The Use of Pit-Fall Traps for Estimating the Abundance of Arthropods, with Special Reference to the Tenebrionidae (Coleoptera). Ann. Entomol. Soc. Am., 70(2):242-248.

Whitkamp, M. 1971. Soils as Components of Ecosystems. Ann. Rev. Ecol. Sys., 2:85-110.

________, and D.A. Crossley, Jr. 1966. The Role of Arthropods and Microflora in Breakdown of White Oak Litter. Pedobiologia, 6:293-303.

APPENDIX TABLE 18.1. 7 X 7 LATIN SQUARE GRID CAPTURE OF *CANTHON* SP. AT EPA ZAPS, 1977

ZAPS I

PLOT A (Control) May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 854	Σ = 606	Σ = 248
$\bar{X}$ = 17.5	$\bar{X}$ = 25.3	$\bar{X}$ = 9.9
S = 21.8	S = 24.9	S = 15.3

		Rows				Columns	
	Σ	$\bar{X}$	S		Σ	$\bar{X}$	S
#1	178	25.4	21.8	#1	220	31.4	34.5
#2	5	0.7	1.3	#2	129	18.4	23.9
#3	35	5.0	4.0	#3	125	17.9	19.5
#4	108	15.4	24.7	#4	79	11.3	14.5
#5	161	23.0	22.5	#5	134	19.1	20.7
#6	117	16.7	26.6	#6	66	9.4	11.8
#7	250	35.7	21.6	#7	101	14.4	22.6

PLOT B (Low) May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 642	Σ = 468	Σ = 174
$\bar{X}$ = 13.1	$\bar{X}$ = 19.5	$\bar{X}$ = 7.0
S = 16.5	S = 19.5	S = 9.9

		Rows				Columns	
	Σ	$\bar{X}$	S		Σ	$\bar{X}$	S
#1	134	19.1	18.4	#1	165	23.6	17.8
#2	55	7.9	15.2	#2	55	7.9	14.2
#3	68	9.7	12.5	#3	142	20.3	20.0
#4	66	9.4	14.1	#4	90	12.9	22.5
#5	39	5.6	5.32	#5	56	8.0	8.4
#6	147	21.0	12.1	#6	49	7.0	13.3
#7	133	19.0	28.1	#7	85	12.1	14.0

ZAPS 1

PLOT C (Medium) May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 631	Σ = 467	Σ = 164
$\bar{X}$ = 12.9	$\bar{X}$ = 19.5	$\bar{X}$ = 6.6
S = 16.8	S = 20.6	S = 8.5

		Rows				Columns	
	Σ	$\bar{X}$	S		Σ	$\bar{X}$	S
#1	106	15.1	17.1	#1	89	12.7	16.3
#2	22	3.1	3.9	#2	95	13.6	15.7
#3	81	11.6	13.0	#3	90	12.9	11.0
#4	10	1.4	1.5	#4	5	0.7	1.3
#5	97	13.9	14.3	#5	38	5.4	5.1
#6	109	15.6	17.8	#6	145	20.7	26.7
#7	206	29.4	25.6	#7	169	24.1	20.7

Plot D (High) May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 908	Σ = 643	Σ = 265
$\bar{X}$ = 18.5	$\bar{X}$ = 26.8	$\bar{X}$ = 10.6
S = 28.1	S = 34.8	S = 17.1

		Rows				Columns	
	Σ	$\bar{X}$	S		Σ	$\bar{X}$	S
#1	162	23.1	28.3	#1	77	11.0	21.4
#2	59	8.4	11.2	#2	135	19.3	23.1
#3	11	1.6	1.8	#3	138	19.7	31.0
#4	97	13.9	18.0	#4	114	16.3	24.2
#5	249	35.6	49.4	#5	62	8.9	8.9
#6	118	16.9	28.0	#6	56	8.0	10.1
#7	212	30.3	29.2	#7	326	46.6	48.9

APPENDIX TABLE 18.1. *CANTHON* SP. (continued)

ZAPS II

PLOT A (Control) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 757	Σ = 531	Σ = 226
$\bar{X}$ = 15.5	$\bar{X}$ = 22.2	$\bar{X}$ = 9.0
S = 18.3	X = 23.1	X = 8.6

	Rows Σ	Rows $\bar{X}$	Rows S		Columns Σ	Columns $\bar{X}$	Columns S
#1	101	14.4	15.3	#1	168	24.0	26.7
#2	129	18.4	26.5	#2	170	24.3	28.3
#3	67	9.6	6.8	#3	117	16.7	19.5
#4	35	5.0	6.8	#4	64	9.1	10.5
#5	70	10.0	8.3	#5	89	12.7	13.9
#6	110	15.7	10.2	#6	45	6.4	6.5
#7	245	35.0	28.9	#7	104	14.9	10.9

PLOT B (Low) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 845	Σ = 709	Σ = 136
$\bar{X}$ = 17.3	$\bar{X}$ = 29.5	$\bar{X}$ = 5.4
S = 28.1	X = 34.9	X = 10.7

	Rows Σ	Rows $\bar{X}$	Rows S		Columns Σ	Columns $\bar{X}$	Columns S
#1	55	7.9	7.6	#1	284	40.6	50.3
#2	114	16.3	20.9	#2	52	7.4	14.2
#3	16	2.3	3.7	#3	130	18.6	27.2
#4	20	2.9	2.1	#4	78	11.1	19.3
#5	17	2.4	2.4	#5	125	17.9	29.6
#6	198	28.3	43.1	#6	41	5.9	8.8
#7	425	60.7	27.1	#7	135	19.3	24.1

ZAPS II

PLOT C (Medium) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 643	Σ = 492	Σ = 151
$\bar{X}$ = 13.1	$\bar{X}$ = 20.5	$\bar{X}$ = 6.0
S = 16.3	S = 19.4	S = 8.1

	Rows Σ	Rows $\bar{X}$	Rows S		Columns Σ	Columns $\bar{X}$	Columns S
#1	123	17.6	14.3	#1	107	15.3	13.3
#2	43	6.1	8.4	#2	101	14.4	26.2
#3	40	5.7	6.3	#3	68	9.7	12.5
#4	47	6.7	5.9	#4	29	4.1	4.1
#5	86	12.3	23.0	#5	81	11.6	9.5
#6	82	11.7	12.6	#6	74	10.6	13.0
#7	222	31.7	22.3	#7	183	26.1	22.6

PLOT D (High) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 966	Σ = 803	Σ = 163
$\bar{X}$ = 19.7	$\bar{X}$ = 33.5	$\bar{X}$ = 6.5
S = 32.5	S = 41.5	S = 9.7

	Rows Σ	Rows $\bar{X}$	Rows S		Columns Σ	Columns $\bar{X}$	Columns S
#1	47	6.7	7.3	#1	53	7.6	6.7
#2	116	16.6	32.6	#2	24	3.4	3.7
#3	95	13.6	24.5	#3	62	8.9	8.7
#4	69	9.9	9.7	#4	95	13.6	26.2
#5	102	14.6	16.1	#5	179	25.6	40.1
#6	106	15.1	24.5	#6	100	14.3	21.8
#7	431	61.6	57.6	#7	453	65.0	49.5

APPENDIX TABLE 18.1. *CANTHON* SP. (continued)

ZAPS I

PLOT A (Control) June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 1,927	Σ = 1,397	Σ = 530
$\overline{X}$ = 39.3	$\overline{X}$ = 58.2	$\overline{X}$ = 21.2
S = 45.7	S = 48.4	S = 35.2

	Rows				Columns		
	Σ	$\overline{X}$	S		Σ	$\overline{X}$	S
#1	520	74.3	49.2	#1	597	85.3	44.6
#2	103	14.7	18.6	#2	215	30.7	43.7
#3	271	38.7	49.4	#3	408	58.3	65.2
#4	243	34.7	54.3	#4	194	27.7	32.5
#5	210	30.0	29.3	#5	167	23.9	38.5
#6	310	44.3	55.7	#6	52	7.4	6.0
#7	270	38.6	48.2	#7	294	42.0	37.5

PLOT B (Low) June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 1,210	Σ = 817	Σ = 393
$\overline{X}$ = 24.7	$\overline{X}$ = 34.0	$\overline{X}$ = 15.7
S = 31.7	S = 38.2	S = 20.9

	Rows				Columns		
	Σ	$\overline{X}$	S		Σ	$\overline{X}$	S
#1	237	33.9	47.8	#1	230	32.9	36.5
#2	232	33.1	40.7	#2	139	19.9	31.2
#3	196	28.0	21.8	#3	240	34.3	38.8
#4	52	7.4	12.8	#4	174	24.9	33.9
#5	103	14.7	18.4	#5	66	9.4	8.8
#6	127	18.1	25.3	#6	66	9.4	16.0
#7	263	35.6	39.6	#7	295	42.1	47.9

ZAPS I

PLOT C (Medium) June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 1,296	Σ = 1,011	Σ = 285
$\overline{X}$ = 26.4	$\overline{X}$ = 42.1	$\overline{X}$ = 11.4
X = 36.7	S = 41.0	S = 24.5

	Rows				Columns		
	Σ	$\overline{X}$	S		Σ	$\overline{X}$	S
#1	385	55.0	51.1	#1	297	42.4	50.9
#2	289	41.3	43.3	#2	151	21.6	38.0
#3	87	12.4	14.2	#3	230	32.9	41.9
#4	17	2.4	2.3	#4	15	2.1	4.0
#5	117	16.7	28.8	#5	70	10.0	10.6
#6	182	26.0	43.1	#6	192	27.4	41.7
#7	219	31.3	34.2	#7	341	48.7	34.2

PLOT D (High) June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 1,644	Σ = 1,556	Σ = 88
$\overline{X}$ = 33.5	$\overline{X}$ = 64.8	$\overline{X}$ = 3.5
S = 46.8	S = 50.1	S = 7.0

	Rows				Columns		
	Σ	$\overline{X}$	S		Σ	$\overline{X}$	S
#1	413	59.0	46.9	#1	200	28.6	40.5
#2	54	7.7	10.5	#2	140	20.0	41.4
#3	106	15.1	36.5	#3	261	37.3	58.2
#4	156	22.3	34.8	#4	115	16.4	39.1
#5	139	19.9	46.0	#5	270	38.6	54.1
#6	106	15.1	37.0	#6	207	29.6	43.5
#7	670	95.7	44.2	#7	451	64.4	51.6

APPENDIX TABLE 18.1. *CANTHON* SP. (continued)

ZAPS II

PLOT A (Control) June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 1,538	Σ = 1,037	Σ = 501
$\overline{X}$ = 31.4	$\overline{X}$ = 43.2	$\overline{X}$ = 20.0
X = 36.4	S = 37.2	S = 32.6

	Rows				Columns		
	Σ	$\overline{X}$	S		Σ	$\overline{X}$	S
#1	195	27.9	20.1	#1	419	59.9	32.4
#2	186	26.6	41.4	#2	495	70.7	48.7
#3	190	27.1	45.0	#3	191	27.3	30.0
#4	166	23.7	40.1	#4	114	16.3	12.3
#5	215	30.7	33.1	#5	106	15.1	34.1
#6	102	14.6	11.7	#6	41	5.9	7.7
#7	484	69.1	39.1	#7	172	24.6	28.2

PLOT B (Low) June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 956	Σ = 858	Σ = 98
$\overline{X}$ = 19.5	$\overline{X}$ = 35.8	$\overline{X}$ = 3.9
S = 31.7	S = 39.0	S = 5.4

	Rows				Columns		
	Σ	$\overline{X}$	S		Σ	$\overline{X}$	S
#1	216	30.9	47.2	#1	273	39.0	35.8
#2	67	9.6	20.1	#2	83	11.9	22.3
#3	39	5.6	8.2	#3	83	11.9	18.5
#4	57	8.1	7.7	#4	93	13.3	28.4
#5	31	4.4	8.0	#5	101	14.4	23.0
#6	56	8.0	8.5	#6	137	19.6	42.6
#7	490	70.0	32.9	#7	186	26.6	45.1

ZAPS II

PLOT C (Medium) June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 895	Σ = 837	Σ = 58
$\overline{X}$ = 18.3	$\overline{X}$ = 34.9	$\overline{X}$ = 2.3
S = 31.9	S = 39.3	S = 4.0

	Rows				Columns		
	Σ	$\overline{X}$	S		Σ	$\overline{X}$	S
#1	95	13.6	29.4	#1	160	22.9	31.2
#2	125	17.9	42.1	#2	122	17.4	36.1
#3	35	5.0	8.6	#3	126	18.0	42.4
#4	18	2.6	3.5	#4	17	2.4	3.0
#5	51	7.3	14.9	#5	86	12.3	27.8
#6	118	16.9	20.5	#6	92	13.1	30.0
#7	453	64.7	40.0	#7	292	41.7	36.8

PLOT D (High) June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 904	Σ = 812	Σ = 92
$\overline{X}$ = 18.5	$\overline{X}$ = 33.8	$\overline{X}$ = 3.7
S = 34.6	S = 44.5	S = 5.7

	Rows				Columns		
	Σ	$\overline{X}$	S		Σ	$\overline{X}$	S
#1	138	19.7	31.8	#1	41	5.9	7.0
#2	24	3.4	3.8	#2	39	5.6	5.6
#3	39	5.6	8.3	#3	42	6.0	8.6
#4	27	3.9	5.7	#4	71	10.1	24.7
#5	154	22.0	44.0	#5	206	29.4	48.5
#6	93	13.3	23.8	#6	121	17.3	43.1
#7	429	61.3	55.1	#7	384	54.9	47.6

APPENDIX TABLE 18.1. *CANTHON* SP. (continued)

ZAPS I

PLOT A (Control)

September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 75	Σ = 63	Σ = 12
$\bar{X}$ = 1.5	$\bar{X}$ = 2.6	$\bar{X}$ = 0.5
S = 2.6	S = 3.3	S = 1.1

	Rows				Columns		
	Σ	$\bar{X}$	S		Σ	$\bar{X}$	S
#1	19	2.7	2.3	#1	39	5.6	4.0
#2	4	0.6	0.8	#2	2	0.3	0.5
#3	7	1.0	2.7	#3	3	0.4	0.8
#4	11	1.6	2.1	#4	8	1.1	2.2
#5	15	2.1	3.9	#5	16	2.3	2.6
#6	11	1.6	3.7	#6	2	0.3	0.5
#7	8	1.2	2.2	#7	5	0.7	1.0

PLOT B (Low)

September 11 - 21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 31	Σ = 28	Σ = 3
$\bar{X}$ = 0.6	$\bar{X}$ = 1.2	$\bar{X}$ = 0.1
S = 1.0	S = 1.3	S = 0.3

	Rows				Columns		
	Σ	$\bar{X}$	S		Σ	$\bar{X}$	S
#1	12	1.7	1.5	#1	9	1.3	1.4
#2	3	0.4	0.5	#2	3	0.4	0.8
#3	4	0.6	0.8	#3	3	0.4	0.8
#4	1	0.1	0.4	#4	6	0.9	1.6
#5	4	0.6	1.5	#5	0	0.0	0.0
#6	2	0.3	0.8	#6	2	0.3	0.5
#7	5	0.7	1.0	#7	8	1.1	1.2

ZAPS I

PLOT C (Medium)

September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 19	Σ = 16	Σ = 3
$\bar{X}$ = 0.4	$\bar{X}$ = 0.7	$\bar{X}$ = 0.1
S = 1.5	S = 2.0	S = 0.3

	Rows				Columns		
	Σ	$\bar{X}$	S		Σ	$\bar{X}$	S
#1	2	0.3	0.5	#1	0	0.0	0.0
#2	3	0.4	0.8	#2	1	0.1	0.4
#3	1	0.1	0.4	#3	10	1.4	3.8
#4	1	0.1	0.4	#4	0	0.0	0.0
#5	1	0.1	0.4	#5	1	0.1	0.4
#6	1	0.1	0.4	#6	3	0.4	0.5
#7	10	1.4	3.8	#7	4	0.6	0.8

PLOT D (High)

September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 49	Σ = 43	Σ = 6.0
$\bar{X}$ = 1.0	$\bar{X}$ = 1.8	$\bar{X}$ = 0.2
S = 3.1	S = 4.3	S = 0.7

	Rows				Columns		
	Σ	$\bar{X}$	S		Σ	$\bar{X}$	S
#1	3	0.4	0.5	#1	14	2.0	3.2
#2	5	0.7	1.3	#2	3	0.4	0.5
#3	1	0.1	0.4	#3	0	0.0	0.0
#4	2	0.3	0.8	#4	3	0.4	0.8
#5	1	0.1	0.4	#5	7	1.0	1.4
#6	22	3.1	7.5	#6	1	0.1	0.4
#7	15	2.1	3.2	#7	21	3.0	7.5

APPENDIX TABLE 18.1. *CANTHON* SP. (continued)

ZAPS II

PLOT A (Control) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 135	Σ = 107	Σ = 28
$\overline{X}$ = 2.7	$\overline{X}$ = 4.5	$\overline{X}$ = 1.1
S = 3.6	S = 4.1	S = 1.8

	Rows				Columns		
	Σ	$\overline{X}$	S		Σ	$\overline{X}$	S
#1	23	3.3	3.3	#1	63	9.0	3.6
#2	19	2.7	5.1	#2	30	4.3	3.5
#3	21	3.0	3.7	#3	11	1.6	1.7
#4	16	2.3	4.8	#4	2	0.3	0.5
#5	14	2.0	3.6	#5	4	0.6	1.1
#6	12	1.7	1.4	#6	13	1.9	1.6
#7	30	4.3	2.5	#7	12	1.7	1.8

PLOT B (Low) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 71	Σ = 45	Σ = 26
$\overline{X}$ = 1.5	$\overline{X}$ = 1.9	$\overline{X}$ = 1.0
S = 2.5	S = 3.1	S = 1.5

	Rows				Columns		
	Σ	$\overline{X}$	S		Σ	$\overline{X}$	S
#1	3	0.4	0.5	#1	10	1.4	1.9
#2	8	1.1	1.9	#2	13	1.9	3.2
#3	2	0.3	0.8	#3	14	2.0	3.2
#4	5	0.7	0.8	#4	3	0.4	0.5
#5	10	1.4	2.1	#5	17	2.4	3.4
#6	8	1.1	1.8	#6	14	2.0	2.5
#7	35	5.0	4.1	#7	0	0.0	0.0

ZAPS II

PLOT C (Medium) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 88	Σ = 72	Σ = 16
$\overline{X}$ = 1.8	$\overline{X}$ = 3.0	$\overline{X}$ = 0.6
S = 2.8	S = 3.6	S = 0.9

	Rows				Columns		
	Σ	$\overline{X}$	S		Σ	$\overline{X}$	S
#1	13	1.9	2.7	#1	6	0.9	1.9
#2	4	0.6	0.8	#2	15	2.1	4.0
#3	2	0.3	0.5	#3	18	2.6	4.0
#4	6	0.9	1.2	#4	5	0.7	1.5
#5	4	0.6	0.8	#5	11	1.6	2.2
#6	9	1.3	1.6	#6	20	2.9	3.6
#7	50	7.14	3.1	#7	13	1.9	1.7

PLOT D (High) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 75	Σ = 70	Σ = 5
$\overline{X}$ = 1.5	$\overline{X}$ = 2.9	$\overline{X}$ = 0.2
S = 2.9	S = 3.6	S = 0.7

	Rows				Columns		
	Σ	$\overline{X}$	S		Σ	$\overline{X}$	S
#1	10	1.4	1.4	#1	7	1.0	1.1
#2	2	0.3	0.5	#2	13	1.9	4.1
#3	2	0.3	0.5	#3	11	1.6	1.6
#4	2	0.3	0.8	#4	14	2.0	5.3
#5	9	1.3	2.6	#5	6	0.9	1.9
#6	5	0.7	1.3	#6	2	0.3	0.5
#7	45	6.4	4.6	#7	22	3.1	2.9

APPENDIX TABLE 18.1. *CANTHON* SP. (continued)

HAY COULEE

Total Traps (28)	Perimeter Traps (18)	Interior Traps (10)
Σ = 195	Σ = 114	Σ = 81
$\overline{X}$ = 6.9	$\overline{X}$ = 6.3	$\overline{X}$ = 8.1
S = 10.0	S = 7.9	S = 13.6

	Rows		
	Σ	$\overline{X}$	S
#1	57	14.2	11.4
#2	11	2.8	2.8
#3	50	12.5	21.7
#4	6	1.5	1.3
#5	7	1.8	2.4
#6	44	11.0	5.0
#7	20	5.0	5.6

	Columns		
	Σ	$\overline{X}$	S
#1	28	4.0	6.2
#2	39	5.6	4.7
#3	69	9.9	15.9
#4	59	8.5	10.9

APPENDIX TABLE 18.2. 7 X 7 LATIN SQUARE GRID CAPTURE OF *ONTHOPHAGUS HECATE* AT EPA ZAPS, 1977

ZAPS I

PLOT A (Control)

May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 216	Σ = 144	Σ = 72
$\bar{X}$ = 4.4	$\bar{X}$ = 6	$\bar{X}$ = 2.9
S = 4.8	S = 5.7	S = 3.1

PLOT B (Low)

May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 172	Σ = 110	Σ = 62
$\bar{X}$ = 3.5	$\bar{X}$ = 4.6	$\bar{X}$ = 2.5
S = 4.4	S = 5	S = 3.6

PLOT C (Medium)

May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 143	Σ = 78	Σ = 65
$\bar{X}$ = 2.9	$\bar{X}$ = 3.3	$\bar{X}$ = 2.6
S = 3.3	S = 3.4	S = 3.2

PLOT D (High)

May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 189	Σ = 117	Σ = 72
$\bar{X}$ = 3.8	$\bar{X}$ = 4.9	$\bar{X}$ = 2.9
S = 4.9	S = 5.7	S = 3.8

ZAPS II

PLOT A (Control)

May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 94	Σ = 44	Σ = 50
$\bar{X}$ = 1.9	$\bar{X}$ = 1.8	$\bar{X}$ = 2.1
S = 2.2	S = 1.9	S = 2.6

PLOT B (Low)

May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 61	Σ = 41	Σ = 20
$\bar{X}$ = 1.2	$\bar{X}$ = 1.7	$\bar{X}$ = .8
S = 2.0	S = 2.1	S = 1.7

PLOT C (Medium)

May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 84	Σ = 57	Σ = 27
$\bar{X}$ = 1.7	$\bar{X}$ = 2.4	$\bar{X}$ = 1.1
S = 1.8	S = 2.1	S = 1.2

PLOT D (High)

May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 156	Σ = 129	Σ = 27
$\bar{X}$ = 3.2	$\bar{X}$ = 5.4	$\bar{X}$ = 1.8
S = 5.5	S = 7	S = 1.6

APPENDIX TABLE 18.2. *ONTHOPHAGUS HECATE* (continued)

ZAPS I

PLOT A (Control) June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 65$	$\Sigma = 52$	$\Sigma = 13$
$\bar{X} = 1.3$	$\bar{X} = 2.2$	$\bar{X} = .5$
$S = 2.2$	$S = 2.9$	$S = .9$

PLOT B (Low) June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 40$	$\Sigma = 30$	$\Sigma = 10$
$\bar{X} = .8$	$\bar{X} = 1.3$	$\bar{X} = .4$
$S = 1.5$	$S = 2.1$	$S = .5$

PLOT C (Medium) June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 60$	$\Sigma = 41$	$\Sigma = 19$
$\bar{X} = 1.2$	$\bar{X} = 1.7$	$\bar{X} = .8$
$S = 2.3$	$S = 1.8$	$S = 2.7$

PLOT D (High) June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 25$	$\Sigma = 25$	$\Sigma = 0$
$\bar{X} = .5$	$\bar{X} = 1$	$\bar{X} = 0$
$S = 1.1$	$S = 1.5$	$S = 0$

ZAPS II

PLOT A (Control) June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 27$	$\Sigma = 21$	$\Sigma = 6$
$\bar{X} = .6$	$\bar{X} = .9$	$\bar{X} = .2$
$S = 1.3$	$S = 1.7$	$S = .4$

PLOT B (Low) June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 6$	$\Sigma = 5$	$\Sigma = 1$
$\bar{X} = .1$	$\bar{X} = .2$	$\bar{X} = 0$
$S = .4$	$S = .5$	$S = .2$

PLOT C (Medium) June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 12$	$\Sigma = 10$	$\Sigma = 2$
$\bar{X} = .2$	$\bar{X} = .4$	$\bar{X} = .1$
$S = .6$	$S = .8$	$S = .3$

PLOT D (High) June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 4$	$\Sigma = 2$	$\Sigma = 2$
$\bar{X} = .08$	$\bar{X} = .1$	$\bar{X} = .1$
$S = .3$	$S = .4$	$S = .3$

APPENDIX TABLE 18.2. *ONTHOPHAGUS HECATE* (continued)

ZAPS I

PLOT A (Control) September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 3$	$\Sigma = 2$	$\Sigma = 1$
$\overline{X} = .06$	$\overline{X} = .1$	$\overline{X} = 0$
$S = .2$	$S = .3$	$S = .2$

PLOT B (Low) September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 4$	$\Sigma = 2$	$\Sigma = 2$
$\overline{X} = .08$	$\overline{X} = .1$	$\overline{X} = .1$
$S = .3$	$S = .3$	$S = .3$

PLOT C (Medium) September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 4$	$\Sigma = 2$	$\Sigma = 2$
$\overline{X} = .08$	$\overline{X} = .1$	$\overline{X} = .1$
$S = .3$	$S = .3$	$S = .3$

PLOT D (High) September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 11$	$\Sigma = 9$	$\Sigma = 2$
$\overline{X} = .2$	$\overline{X} = .4$	$\overline{X} = .1$
$S = .6$	$S = .8$	$S = .3$

ZAPS II

PLOT A (Control) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 19$	$\Sigma = 10$	$\Sigma = 9$
$\overline{X} = .4$	$\overline{X} = .4$	$\overline{X} = .4$
$S = .6$	$S = .6$	$S = .6$

PLOT B (Low) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 17$	$\Sigma = 11$	$\Sigma = 6$
$\overline{X} = .4$	$\overline{X} = .5$	$\overline{X} = .2$
$S = .8$	$S = .8$	$S = .7$

PLOT C (Medium) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 17$	$\Sigma = 12$	$\Sigma = 5$
$\overline{X} = .4$	$\overline{X} = .5$	$\overline{X} = .2$
$S = .7$	$S = .8$	$S = .4$

PLOT D (High) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 15$	$\Sigma = 12$	$\Sigma = 3$
$\overline{X} = .3$	$\overline{X} = .5$	$\overline{X} = .12$
$S = .6$	$S = .7$	$S = .33$

APPENDIX TABLE 18.3. 7 X 7 LATIN SQUARE GRID CAPTURE OF *NICROPHORUS* SP. AT EPA ZAPS, 1977

ZAPS I

PLOT A (Control) May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 60$	$\Sigma = 36$	$\Sigma = 24$
$\overline{X} = 1.2$	$\overline{X} = 1.5$	$\overline{X} = 1$
$S = 1.7$	$S = 2$	$S = 1.5$

PLOT B (Low) May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 34$	$\Sigma = 19$	$\Sigma = 15$
$\overline{X} = .7$	$\overline{X} = .8$	$\overline{X} = .6$
$S = 1.3$	$S = 1.2$	$S = 1.3$

PLOT C (Medium) May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 43$	$\Sigma = 23$	$\Sigma = 20$
$\overline{X} = .9$	$\overline{X} = 1$	$\overline{X} = .8$
$S = 1.2$	$S = 1.1$	$S = 1.2$

PLOT D (High) May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 53$	$\Sigma = 38$	$\Sigma = 15$
$\overline{X} = 1.1$	$\overline{X} = 1.6$	$\overline{X} = .6$
$S = 1.8$	$S = 2.3$	$S = .9$

ZAPS II

PLOT A (Control) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 39$	$\Sigma = 25$	$\Sigma = 14$
$\overline{X} = .8$	$\overline{X} = 1$	$\overline{X} = .6$
$S = 1.3$	$S = 1.3$	$S = 1.2$

PLOT B (Low) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 55$	$\Sigma = 44$	$\Sigma = 11$
$\overline{X} = 1.1$	$\overline{X} = 1.8$	$\overline{X} = .4$
$S = 2.6$	$S = 3.5$	$S = .9$

PLOT C (Medium) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 29$	$\Sigma = 20$	$\Sigma = 9$
$\overline{X} = .6$	$\overline{X} = .8$	$\overline{X} = .4$
$S = .8$	$S = .8$	$S = .6$

PLOT D (High) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 74$	$\Sigma = 66$	$\Sigma = 8$
$\overline{X} = 1.5$	$\overline{X} = 2.8$	$\overline{X} = .3$
$S = 3$	$S = 3.9$	$S = .6$

APPENDIX TABLE 18.3. *NICROPHORUS* SP. (continued)

ZAPS I

PLOT A (Control) June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 65$	$\Sigma = 51$	$\Sigma = 14$
$\bar{X} = 1.3$	$\bar{X} = 2.1$	$\bar{X} = 1.6$
$S = 1.9$	$S = 2.4$	$S = 1$

PLOT B (Low) June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 32$	$\Sigma = 22$	$\Sigma = 10$
$\bar{X} = .7$	$\bar{X} = .9$	$\bar{X} = .4$
$S = 1$	$S = 1.1$	$S = .8$

PLOT C (Medium) June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 35$	$\Sigma = 29$	$\Sigma = 6$
$\bar{X} = .7$	$\bar{X} = 1.2$	$\bar{X} = .2$
$S = 1.4$	$S = 1.8$	$S = .7$

PLOT D (High) June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 35$	$\Sigma = 29$	$\Sigma = 6$
$\bar{X} = .7$	$\bar{X} = 1.2$	$\bar{X} = .1$
$S = 1.1$	$S = 1.4$	$S = .4$

ZAPS II

PLOT A (Control) June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 66$	$\Sigma = 45$	$\Sigma = 21$
$\bar{X} = 1.4$	$\bar{X} = 1.9$	$\bar{X} = 1.8$
$S = 1.9$	$S = 1.9$	$S = 1.8$

PLOT B (Low) June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 39$	$\Sigma = 21$	$\Sigma = 18$
$\bar{X} = .8$	$\bar{X} = .9$	$\bar{X} = .7$
$S = 1.6$	$S = 1.3$	$S = 1.8$

PLOT C (Medium) June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 39$	$\Sigma = 31$	$\Sigma = 8$
$\bar{X} = .8$	$\bar{X} = 1.3$	$\bar{X} = .3$
$S = 1.2$	$S = 1.3$	$S = .8$

PLOT D (High) June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 144$	$\Sigma = 110$	$\Sigma = 34$
$\bar{X} = 2.9$	$\bar{X} = 4.6$	$\bar{X} = 1.4$
$S = 4.4$	$S = 5.3$	$S = 2.6$

APPENDIX TABLE 18.3. *NICROPHORUS* SP. (continued)

ZAPS I

PLOT A (Control) September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 37$	$\Sigma = 31$	$\Sigma = 6$
$\bar{X} = .8$	$\bar{X} = 1.3$	$\bar{X} = .2$
$S = .5$	$S = 1.4$	$S = .5$

PLOT B (Low) September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 24$	$\Sigma = 20$	$\Sigma = 4$
$\bar{X} = .5$	$\bar{X} = .8$	$\bar{X} = .2$
$S = 1.2$	$S = 1.6$	$S = .4$

PLOT C (Medium) September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 43$	$\Sigma = 30$	$\Sigma = 13$
$\bar{X} = .9$	$\bar{X} = 1.3$	$\bar{X} = .5$
$S = 1.3$	$S = 1.6$	$S = .9$

PLOT D (High) September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 33$	$\Sigma = 28$	$\Sigma = 5$
$\bar{X} = .7$	$\bar{X} = 1.2$	$\bar{X} = .2$
$S = 1$	$S = 1.1$	$S = .5$

ZAPS II

PLOT A (Control) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 76$	$\Sigma = 54$	$\Sigma = 22$
$\bar{X} = 1.6$	$\bar{X} = 2.3$	$\bar{X} = .9$
$S = 2.6$	$S = 3.3$	$S = 1.3$

PLOT B (Low) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 41$	$\Sigma = 26$	$\Sigma = 15$
$\bar{X} = .8$	$\bar{X} = 1.1$	$\bar{X} = .6$
$S = 1.2$	$S = 1.4$	$S = .9$

PLOT C (Medium) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 38$	$\Sigma = 31$	$\Sigma = 7$
$\bar{X} = .8$	$\bar{X} = 1.3$	$\bar{X} = .3$
$S = 1.4$	$S = 1.8$	$S = .5$

PLOT D (High) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 69$	$\Sigma = 53$	$\Sigma = 16$
$\bar{X} = 1.4$	$\bar{X} = 2.2$	$\bar{X} = .6$
$S = 2.2$	$S = 2.8$	$S = .9$

APPENDIX TABLE 18.4. 7 X 7 LATIN SQUARE GRID CAPTURE OF *TROX* SP. AT EPA ZAPS, 1977

ZAPS I

PLOT A (Control) May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 7	Σ = 3	Σ = 4
$\bar{X}$ = .1	$\bar{X}$ = .1	$\bar{X}$ = .2
S = .4	S = .3	S = .4

PLOT B (Low) May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 8	Σ = 4	Σ = 4
$\bar{X}$ = .2	$\bar{X}$ = .2	$\bar{X}$ = .2
S = .4	S = .4	S = .5

PLOT C (Medium) May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 6	Σ = 3	Σ = 3
$\bar{X}$ = .1	$\bar{X}$ = .1	$\bar{X}$ = .1
S = .3	S = .3	S = .3

PLOT D (High) May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 5	Σ = 2	Σ = 3
$\bar{X}$ = .1	$\bar{X}$ = .1	$\bar{X}$ = .1
S = .3	S = .3	S = .3

ZAPS II

PLOT A (Control) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 10	Σ = 2	Σ = 8
$\bar{X}$ = .2	$\bar{X}$ = .1	$\bar{X}$ = .3
S = .5	S = .4	S = .6

PLOT B (Low) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 20	Σ = 9	Σ = 11
$\bar{X}$ = .4	$\bar{X}$ = .4	$\bar{X}$ = .4
S = .8	S = .7	S = .9

PLOT C (Medium) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 9	Σ = 9	Σ = 0
$\bar{X}$ = .2	$\bar{X}$ = .4	$\bar{X}$ = 0
S = .5	S = .7	S = 0

PLOT D (High) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 25	Σ = 22	Σ = 3
$\bar{X}$ = .5	$\bar{X}$ = .9	$\bar{X}$ = .1
S = 1.3	S = 1.7	S = .3

APPENDIX TABLE 18.4. *TROX* SP. (continued)

ZAPS I

PLOT A (Control) June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 4	Σ = 2	Σ = 2
$\bar{X}$ = .1	$\bar{X}$ = .1	$\bar{X}$ = .1
S = .3	S = .3	S = .3

PLOT B (Low) June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 20	Σ = 20	Σ = 0
$\bar{X}$ = .4	$\bar{X}$ = .8	$\bar{X}$ = 0
S = 2.4	S = 3.5	S = 0

PLOT C (Medium) June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 1	Σ = 1	Σ = 0
$\bar{X}$ = .02	$\bar{X}$ = .1	$\bar{X}$ = 0
S = .1	S = .2	S = 0

PLOT D (High) June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 2	Σ = 2	Σ = 0
$\bar{X}$ = .04	$\bar{X}$ = .1	$\bar{X}$ = 0
S = .2	S = .3	S = 0

ZAPS II

PLOT A (Control) June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 8	Σ = 1	Σ = 7
$\bar{X}$ = .2	$\bar{X}$ = .1	$\bar{X}$ = .3
S = .9	S = .2	S = 1.2

PLOT B (Low) June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 3	Σ = 0	Σ = 3
$\bar{X}$ = .06	$\bar{X}$ = 0	$\bar{X}$ = .1
S = .3	S = 0	S = .5

PLOT C (Medium) June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 2	Σ = 1	Σ = 1
$\bar{X}$ = .04	$\bar{X}$ = .1	$\bar{X}$ = .1
S = .2	S = .2	S = .2

PLOT D (High) June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 6	Σ = 5	Σ = 1
$\bar{X}$ = .1	$\bar{X}$ = .2	$\bar{X}$ = .1
S = .7	S = .1	S = .2

APPENDIX TABLE 18.4. *TROX* SP. (continued)

ZAPS I

PLOT A (Control) September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 58$	$\Sigma = 47$	$\Sigma = 11$
$\bar{X} = 1.2$	$\bar{X} = 2$	$\bar{X} = .4$
$S = 1.9$	$S = 2.3$	$S = 1.1$

PLOT B (Low) September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 21$	$\Sigma = 15$	$\Sigma = 6$
$\bar{X} = .4$	$\bar{X} = .6$	$\bar{X} = .2$
$S = 1.1$	$S = 1.4$	$S = .8$

PLOT C (Medium) September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 21$	$\Sigma = 19$	$\Sigma = 2$
$\bar{X} = .4$	$\bar{X} = .8$	$\bar{X} = .1$
$S = .9$	$S = 1.1$	$S = .3$

PLOT D (High) September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 36$	$\Sigma = 27$	$\Sigma = 9$
$\bar{X} = .7$	$\bar{X} = 1.1$	$\bar{X} = .4$
$S = 1.2$	$S = 1.5$	$S = .8$

ZAPS II

PLOT A (Control) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 58$	$\Sigma = 39$	$\Sigma = 19$
$\bar{X} = 1.2$	$\bar{X} = 1.6$	$\bar{X} = .8$
$S = 1.8$	$S = 1.7$	$S = 1.7$

PLOT B (Low) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 22$	$\Sigma = 12$	$\Sigma = 10$
$\bar{X} = .5$	$\bar{X} = .5$	$\bar{X} = .4$
$S = 1.0$	$S = 1.3$	$S = .7$

PLOT C (Medium) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 16$	$\Sigma = 9$	$\Sigma = 7$
$\bar{X} = .3$	$\bar{X} = .4$	$\bar{X} = .3$
$S = .7$	$S = .8$	$S = .6$

PLOT D (High) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 44$	$\Sigma = 31$	$\Sigma = 13$
$\bar{X} = .9$	$\bar{X} = 1.3$	$\bar{X} = .5$
$S = 1.6$	$S = 2$	$S = 1$

APPENDIX TABLE 18.5. 7 X 7 LATIN SQUARE GRID CAPTURE OF *PASIMACHUS ELONGATUS* AT EPA ZAPS, 1977

ZAPS I

PLOT A (Control) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 4$	$\Sigma = 4$	$\Sigma = 0$
$\bar{X} = .08$	$\bar{X} = .2$	$\bar{X} = 0$
$S = .3$	$S = .4$	$S = 0$

PLOT B (Low) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 28$	$\Sigma = 16$	$\Sigma = 12$
$\bar{X} = .6$	$\bar{X} = .7$	$\bar{X} = .5$
$S = 1$	$S = 1.2$	$S = .7$

PLOT C (Medium) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 17$	$\Sigma = 8$	$\Sigma = 9$
$\bar{X} = .4$	$\bar{X} = .3$	$\bar{X} = .4$
$S = .7$	$S = .7$	$S = .8$

PLOT D (High) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 19$	$\Sigma = 6$	$\Sigma = 13$
$\bar{X} = .4$	$\bar{X} = .3$	$\bar{X} = .5$
$S = .8$	$S = .6$	$S = .9$

ZAPS II

PLOT A (Control) May 11-21, 197

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 7$	$\Sigma = 4$	$\Sigma = 3$
$\bar{X} = .1$	$\bar{X} = .2$	$\bar{X} = .1$
$S = .4$	$S = .4$	$S = .5$

PLOT B (Low) May 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 13$	$\Sigma = 12$	$\Sigma = 1$
$\bar{X} = .3$	$\bar{X} = .5$	$\bar{X} = .04$
$S = .6$	$S = .8$	$S = .2$

PLOT C (Medium) May 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 11$	$\Sigma = 8$	$\Sigma = 3$
$\bar{X} = .2$	$\bar{X} = .3$	$\bar{X} = .1$
$S = .6$	$S = .7$	$S = .33$

PLOT D (High) May 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 10$	$\Sigma = 10$	$\Sigma = 0$
$\bar{X} = .2$	$\bar{X} = .4$	$\bar{X} = 0$
$S = .6$	$S = .8$	$S = 0$

APPENDIX TABLE 18.5. *PASIMACHUS ELONGATUS* (continued)

ZAPS I

PLOT A (Control) June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 23$	$\Sigma = 12$	$\Sigma = 11$
$\overline{X} = .5$	$\overline{X} = .5$	$\overline{X} = .4$
$S = .9$	$S = 1$	$S = .7$

PLOT B (Low) June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 7$	$\Sigma = 4$	$\Sigma = 3$
$\overline{X} = .1$	$\overline{X} = .2$	$\overline{X} = .1$
$S = .4$	$S = .4$	$S = .3$

PLOT C (Medium) June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 15$	$\Sigma = 10$	$\Sigma = 5$
$\overline{X} = .3$	$\overline{X} = .4$	$\overline{X} = .2$
$S = .6$	$S = .8$	$S = .4$

PLOT D (High) June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 12$	$\Sigma = 9$	$\Sigma = 3$
$\overline{X} = .2$	$\overline{X} = .4$	$\overline{X} = .1$
$S = .5$	$S = .7$	$S = .3$

ZAPS II

PLOT A (Control) June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 11$	$\Sigma = 5$	$\Sigma = 6$
$\overline{X} = .2$	$\overline{X} = .2$	$\overline{X} = .2$
$S = .6$	$S = .3$	$S = .7$

PLOT B (Low) June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 18$	$\Sigma = 13$	$\Sigma = 5$
$X = .4$	$X = .5$	$X = .2$
$S = .8$	$S = 1$	$S = .5$

PLOT C (Medium) June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 13$	$\Sigma = 10$	$\Sigma = 3$
$\overline{X} = .3$	$\overline{X} = .4$	$\overline{X} = .1$
$S = .5$	$S = .6$	$S = .3$

PLOT D (High) June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 17$	$\Sigma = 13$	$\Sigma = 4$
$\overline{X} = .4$	$\overline{X} = .5$	$\overline{X} = .2$
$S = .9$	$S = 1.2$	$S = .5$

APPENDIX TABLE 18.5. *PASIMACHUS ELONGATUS* (continued)

ZAPS I

PLOT A (Control) — September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 2$	$\Sigma = 1$	$\Sigma = 1$
$\bar{X} = .04$	$\bar{X} = .04$	$\bar{X} = .04$
$S = .2$	$S = .2$	$S = .2$

PLOT B (Low) — September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 2$	$\Sigma = 1$	$\Sigma = 1$
$\bar{X} = .04$	$\bar{X} = .04$	$\bar{X} = .04$
$S = .2$	$S = .2$	$S = .2$

PLOT C (Medium) — September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 2$	$\Sigma = 2$	$\Sigma = 0$
$\bar{X} = .04$	$\bar{X} = .08$	$\bar{X} = 0$
$S = .2$	$S = .3$	$S = 0$

PLOT D (High) — September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 3$	$\Sigma = 2$	$\Sigma = 1$
$\bar{X} = .06$	$\bar{X} = .08$	$\bar{X} = .04$
$S = .2$	$S = .3$	$S = .2$

ZAPS II

PLOT A (Control) — September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 2$	$\Sigma = 0$	$\Sigma = 2$
$\bar{X} = .04$	$\bar{X} = 0$	$\bar{X} = .08$
$S = .2$	$S = 0$	$S = .3$

PLOT B (Low) — September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 15$	$\Sigma = 7$	$\Sigma = 8$
$\bar{X} = .3$	$\bar{X} = .3$	$\bar{X} = .3$
$S = .8$	$S = .9$	$S = .7$

PLOT C (Medium) — September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 5$	$\Sigma = 1$	$\Sigma = 4$
$\bar{X} = .1$	$\bar{X} = .04$	$\bar{X} = .2$
$S = .4$	$S = .2$	$S = .5$

PLOT D (High) — September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 7$	$\Sigma = 5$	$\Sigma = 2$
$\bar{X} = .1$	$\bar{X} = .2$	$\bar{X} = .08$
$S = .4$	$S = .5$	$S = .3$

APPENDIX TABLE 18.6. 7 X 7 LATIN SQUARE GRID CAPTURE OF CURCULIONIDAE (WEEVIL) AT EPA ZAPS, 1977

ZAPS I

PLOT A (Contr.

May 10-11, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 0$	$\Sigma = 0$	$\Sigma = 0$
$\bar{X} = 0$	$\bar{X} = 0$	$\bar{X} = 0$
$S = 0$	$S = 0$	$S = 0$

PLOT B (Low)

May 10-11, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 3$	$\Sigma = 2$	$\Sigma = 1$
$\bar{X} = .06$	$\bar{X} = .08$	$\bar{X} = .04$
$S = .2$	$S = .3$	$S = .2$

PLOT C (Medium)

May 10-11, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 4$	$\Sigma = 4$	$\Sigma = 0$
$\bar{X} = .08$	$\bar{X} = .2$	$\bar{X} = 0$
$S = .3$	$S = .4$	$S = 0$

PLOT D (High)

May 10-11, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 0$	$\Sigma = 0$	$\Sigma = 0$
$\bar{X} = 0$	$\bar{X} = 0$	$\bar{X} = 0$
$S = 0$	$S = 0$	$S = 0$

ZAPS II

PLOT A (Control)

May 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 18$	$\Sigma = 15$	$\Sigma = 3$
$\bar{X} = .4$	$\bar{X} = .6$	$\bar{X} = .1$
$S = .8$	$S = 1$	$S = .3$

PLOT B (Low)

May 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 4$	$\Sigma = 3$	$\Sigma = 1$
$\bar{X} = .08$	$\bar{X} = .1$	$\bar{X} = .04$
$S = .3$	$S = .3$	$S = .2$

PLOT C (Medium)

May 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 8$	$\Sigma = 6$	$\Sigma = 2$
$\bar{X} = .2$	$\bar{X} = .3$	$\bar{X} = .08$
$S = .6$	$S = .7$	$S = .3$

PLOT D (High)

May 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 13$	$\Sigma = 8$	$\Sigma = 5$
$\bar{X} = .3$	$\bar{X} = .3$	$\bar{X} = .2$
$S = .7$	$S = .8$	$S = .6$

APPENDIX TABLE 18.6. CURCULIONIDAE (WEEVIL) (continued)

NO CAPTURES: ZAPS I, June 22-July 2, 1977

ZAPS II, June 23-July 3, 1977

ZAPS II, September 10-20, 1977

ZAPS I

PLOT A (Control) — **September 11-21, 1977**

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 0$	$\Sigma = 0$	$\Sigma = 0$
$\bar{X} = 0$	$\bar{X} = 0$	$\bar{X} = 0$
$S = 0$	$S = 0$	$S = 0$

PLOT B (Low) — **September 11-21, 1977**

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 0$	$\Sigma = 0$	$\Sigma = 0$
$\bar{X} = 0$	$\bar{X} = 0$	$\bar{X} = 0$
$S = 0$	$S = 0$	$S = 0$

PLOT C (Medium) — **September 11-21, 1977**

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 0$	$\Sigma = 0$	$\Sigma = 0$
$\bar{X} = 0$	$\bar{X} = 0$	$\bar{X} = 0$
$S = 0$	$S = 0$	$S = 0$

PLOT D (High) — **September 11-21, 1977**

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 1$	$\Sigma = 0$	$\Sigma = 1$
$\bar{X} = .02$	$\bar{X} = 0$	$\bar{X} = .04$
$S = .14$	$S = 0$	$S = .2$

APPENDIX TABLE 18.7. 7 X 7 LATIN SQUARE GRID CAPTURE OF *CICINDELA* SP. AT EPA ZAPS, 1977

ZAPS I

PLOT A (Control) May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 9	Σ = 7	Σ = 2
$\overline{X}$ = .18	$\overline{X}$ = .3	$\overline{X}$ = .08
S = .91	S = 1.2	S = .4

PLOT B (Low) May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 19	Σ = 15	Σ = 4
$\overline{X}$ = .4	$\overline{X}$ = .63	$\overline{X}$ = .2
S = 1.1	S = 1.6	S = .5

PLOT C (Medium) May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 6	Σ = 4	Σ = 2
$\overline{X}$ = .1	$\overline{X}$ = .2	$\overline{X}$ = .08
S = .4	S = .5	S = .3

PLOT D (High) May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 6	Σ = 1	Σ = 5
$\overline{X}$ = .1	$\overline{X}$ = .04	$\overline{X}$ = .2
S = .4	S = .2	S = .4

ZAPS II

PLOT A (Control) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 6	Σ = 3	Σ = 3
$\overline{X}$ = .1	$\overline{X}$ = .1	$\overline{X}$ = .1
S = .4	S = .3	S = .3

PLOT B (Low) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 2	Σ = 2	Σ = 0
$\overline{X}$ = .04	$\overline{X}$ = .1	$\overline{X}$ = 0
S = .2	S = .3	S = 0

PLOT C (Medium) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 8	Σ = 5	Σ = 3
$\overline{X}$ = .2	$\overline{X}$ = .2	$\overline{X}$ = .1
S = .5	S = .5	S = .4

PLOT D (High) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 5	Σ = 3	Σ = 2
$\overline{X}$ = .1	$\overline{X}$ = .1	$\overline{X}$ = .04
S = .3	S = .3	S = .3

APPENDIX TABLE 18.7. *CICINDELA* SP. (continued)

NO CAPTURES: ZAPS I and II, June 22-July 3, 1977

ZAPS I

PLOT A (Control) September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 2$	$\Sigma = 2$	$\Sigma = 0$
$\bar{X} = .04$	$\bar{X} = .04$	$\bar{X} = 0$
$S = .2$	$S = .3$	$S = 0$

PLOT B (Low) September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 2$	$\Sigma = 2$	$\Sigma = 0$
$\bar{X} = .04$	$\bar{X} = .08$	$\bar{X} = 0$
$S = .2$	$S = .4$	$S = 0$

PLOT C (Medium) September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 2$	$\Sigma = 1$	$\Sigma = 1$
$\bar{X} = .04$	$\bar{X} = .04$	$\bar{X} = .04$
$S = .2$	$S = .2$	$S = .2$

PLOT D (High) September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 2$	$\Sigma = 0$	$\Sigma = 2$
$\bar{X} = .04$	$\bar{X} = 0$	$\bar{X} = .08$
$S = .2$	$S = 0$	$S = .3$

ZAPS II

PLOT A (Control) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 1$	$\Sigma = 1$	$\Sigma = 0$
$X = .04$	$X = .04$	$X = 0$
$S = .2$	$S = .2$	$X = 0$

PLOT B (Low) Septmeber 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 3$	$\Sigma = 2$	$\Sigma = 1$
$\bar{X} = .06$	$\bar{X} = .08$	$\bar{X} = .04$
$S = .2$	$S = .3$	$S = .2$

PLOT C (Medium) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 3$	$\Sigma = 3$	$\Sigma = 0$
$\bar{X} = .06$	$\bar{X} = .1$	$\bar{X} = 0$
$S = .2$	$S = .3$	$S = 0$

PLOT D (High) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 5$	$\Sigma = 4$	$\Sigma = 1$
$\bar{X} = .1$	$\bar{X} = .2$	$\bar{X} = .04$
$S = .3$	$S = .8$	$S = .2$

APPENDIX TABLE 18.8. 7 X 7 LATIN SQUARE GRID CAPTURE OF OTHER BEETLES AT EPA ZAPS, 1977

ZAPS I

PLOT A (Control) May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 62	Σ = 25	Σ = 37
$\bar{X}$ = 1.3	$\bar{X}$ = 1.04	$\bar{X}$ = 1.5
S = 1.9	S = 1.8	S = 2.0

PLOT B (Low) May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 132	Σ = 69	Σ = 63
$\bar{X}$ = 2.7	$\bar{X}$ = 2.9	$\bar{X}$ = 2.5
S = 2.4	S = 2.4	S = 2.6

PLOT C (Medium) May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 146	Σ = 75	Σ = 71
$\bar{X}$ = 2.98	$\bar{X}$ = 3.8	$\bar{X}$ = 2.8
S = 3.34	S = 3.4	S = 2.9

PLOT D (High) May 9-19, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 102	Σ = 53	Σ = 49
$\bar{X}$ = 2	$\bar{X}$ = 2.2	$\bar{X}$ = 2.0
S = 2.5	S = 3.2	S = 1.7

ZAPS II

PLOT A (Control) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 287	Σ = 184	Σ = 103
$\bar{X}$ = 5.9	$\bar{X}$ = 7.4	$\bar{X}$ = 4.1
S = 7.6	S = 9.1	S = 5.2

PLOT B (Low) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 37	Σ = 19	Σ = 18
$\bar{X}$ = .7	$\bar{X}$ = .8	$\bar{X}$ = .7
S = 1.4	S = 1.4	S = 1.3

PLOT C (Medium) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 95	Σ = 54	Σ = 41
$\bar{X}$ = 1.94	$\bar{X}$ = 2.3	$\bar{X}$ = 1.6
S = 1.78	S = 2	S = 1.5

PLOT D (High) May 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
Σ = 222	Σ = 147	Σ = 75
$\bar{X}$ = 4.53	$\bar{X}$ = 6.1	$\bar{X}$ = 3
S = 4.76	S = 5.8	S = 2.9

APPENDIX TABLE 18.8. OTHER BEETLES (continued)

ZAPS I

PLOT A (Control) — June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 17$	$\Sigma = 10$	$\Sigma = 7$
$\bar{X} = .35$	$\bar{X} = .4$	$\bar{X} = .3$
$S = .69$	$S = .8$	$S = .6$

PLOT B (Low) — June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 44$	$\Sigma = 19$	$\Sigma = 25$
$\bar{X} = .9$	$\bar{X} = .8$	$\bar{X} = 1$
$S = 1.2$	$S = 1.1$	$S = 1.3$

PLOT C (Medium) — June 22-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 45$	$\Sigma = 11$	$\Sigma = 34$
$\bar{X} = .9$	$\bar{X} = .5$	$\bar{X} = 1.4$
$S = 1.1$	$S = .6$	$S = 1.2$

PLOT D (High) — June 2-July 2, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 18$	$\Sigma = 15$	$\Sigma = 3$
$\bar{X} = .4$	$\bar{X} = .6$	$\bar{X} = .12$
$S = .9$	$S = 1.2$	$S = .3$

ZAPS II

PLOT A (Control) — June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 21$	$\Sigma = 7$	$\Sigma = 14$
$\bar{X} = .4$	$\bar{X} = .3$	$\bar{X} = .56$
$S = 1.1$	$S = .5$	$S = 1.5$

PLOT B (Low) — June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 7$	$\Sigma = 4$	$\Sigma = 3$
$\bar{X} = .14$	$\bar{X} = .2$	$\bar{X} = .12$
$S = .35$	$S = .4$	$S = .3$

PLOT C (Medium) — June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 17$	$\Sigma = 8$	$\Sigma = 9$
$\bar{X} = .35$	$\bar{X} = .3$	$\bar{X} = .4$
$S = .75$	$S = .7$	$S = .8$

PLOT D (High) — June 23-July 3, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 78$	$\Sigma = 47$	$\Sigma = 31$
$\bar{X} = 1.6$	$\bar{X} = 2$	$\bar{X} = 1.2$
$S = 2.2$	$S = 2.05$	$S = 2.4$

APPENDIX TABLE 18.8. OTHER BEETLES (continued)

ZAPS I

PLOT A (Control) September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 3$	$\Sigma = 1$	$\Sigma = 2$
$\bar{X} = .06$	$\bar{X} = .04$	$\bar{X} = .08$
$S = .2$	$S = .2$	$S = .3$

PLOT B (Low) September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 6$	$\Sigma = 5$	$\Sigma = 1$
$\bar{X} = .1$	$\bar{X} = .2$	$\bar{X} = .04$
$S = .3$	$S = .4$	$S = .2$

PLOT C (Medium) September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 8$	$\Sigma = 4$	$\Sigma = 4$
$\bar{X} = .2$	$\bar{X} = .2$	$\bar{X} = .2$
$S = .4$	$S = .4$	$S = .4$

PLOT D (High) September 11-21, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 9$	$\Sigma = 5$	$\Sigma = 4$
$\bar{X} = .2$	$\bar{X} = .2$	$\bar{X} = .2$
$S = .4$	$S = .4$	$S = .4$

ZAPS II

PLOT A (Control) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 10$	$\Sigma = 7$	$\Sigma = 3$
$\bar{X} = .2$	$\bar{X} = .3$	$\bar{X} = .1$
$S = .5$	$S = .6$	$S = .3$

PLOT B (Low) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 7$	$\Sigma = 4$	$\Sigma = 3$
$\bar{X} = .1$	$\bar{X} = .2$	$\bar{X} = .1$
$S = .4$	$S = .5$	$S = .3$

PLOT C (Medium) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 8$	$\Sigma = 3$	$\Sigma = 5$
$\bar{X} = .2$	$\bar{X} = .1$	$\bar{X} = .2$
$S = .5$	$S = .5$	$S = .6$

PLOT D (High) September 10-20, 1977

Total Traps (49)	Perimeter Traps (24)	Interior Traps (25)
$\Sigma = 2$	$\Sigma = 0$	$\Sigma = 2$
$\bar{X} = .04$	$\bar{X} = 0$	$\bar{X} = .08$
$S = .2$	$S = 0$	$S = .3$

APPENDIX TABLE 18.9. CHI-SQUARE GOODNESS OF FIT FOR PITFALL CAPTURE OF *CANTHON* SP. AT ZAPS, 1977

MAY

ZAPS I	Total X^2			Interior X^2		
	N.S.	P <0.05	P <0.005	N.S.	P <0.05	P <0.005
ABCD			80.79			36.90
AB			30.04			12.98
A C			33.48			17.13
A D	1.66			0.56		
BC	0.10			0.30		
B D			45.65			18.86
CD			49.86			23.78
ZAPS II						
ABCD			70.67			24.13
AB		5.54				19.82
A C			8.37			12.83
A D			26.92			8.48
BC			27.42	0.78		
B D			8.08	2.44		
CD			64.84	0.46		

JUNE-JULY

ZAPS I	Total X^2			Interior X^2		
	N.S.	P <0.05	P <0.005	N.S.	P <0.05	P <0.005
ABCD			215.43			322.27
AB			163.88			20.33
A C			123.54			73.65
A D			22.43			316.12
BC	2.95					17.20
B D			66.00			193.40
CD			41.19			104.05
ZAPS II						
ABCD			270.36			705.92
AB			135.82			271.13
A C			169.93			351.07
A D			164.60			282.09
BC	2.01					10.26
B D	1.45			0.19		
CD	0.05				7.71	

SEPTEMBER

ZAPS I	Total X^2			Interior X^2		
	N.S.	P <0.05	P <0.005	N.S.	P <0.05	P <0.005
ABCD			40.90			9.00
AB			18.26		5.4	
A C			33.40		5.4	
A D		5.45		2.00		
BC	2.88			0.00		
B D		4.05		1.00		
CD	3.57			1.00		
ZAPS II						
ABCD			28.13			17.85
AB			19.88	0.07		
A C			9.91	3.27		
A D			17.14			14.67
BC	1.82			2.38		
B D	0.11					14.23
CD	1.04				5.76	

SECTION 19

EFFECTS OF CONTROLLED LEVELS OF SO_2 ON INVERTEBRATE CONSUMERS

J. W. Leetham, T. J. McNary and J. L. Dodd

ABSTRACT

Presented in this section are summarizations to date of the various studies of the effects of chronic, low level SO_2 exposure on the invertebrate consumers in the ZAPS plots. Included are season summaries for the 1975 and 1976 field censusing for aboveground arthropods, soil macroarthropods and microarthropods, results of the single mid-season samplings for soil microarthropods (1977), nematodes (1976, 1977), tardigrades (1977) and rotifers (1977), and summary of the intensified studies on grasshoppers. A majority of the groups discussed failed to show substantial population level treatment responses. However, population reductions with increasing SO_2 were noted in the Coleoptera families, Curculionidae and Carabidae, and the Lepidoptera family, Pyralidae (larvae), and grasshopper adults (Acrididae). Also, population reductions were noted in the high treatment plots in both ZAPS for both the tardigrades and rotifers. The soil microarthropods showed highest populations in the medium and low treatment plots with the lowest populations under the pipe orifaces of the high treatment (1977 data only). Controlled feeding trials in laboratory cages showed two grasshopper species, *Aulocara elliotti* and *Agenotettix deorum*, selectively rejected western wheatgrass (*Agropyron smithii*) that had been previously exposed to SO_2 on the high ZAPS treatment.

BIOMASS AND POPULATION DYNAMICS OF INVERTEBRATE FAUNA

The total arthropod fauna on the control and all treatments of both ZAPS sites was censused during the 1975 and 1976 growing seasons (1976 only for ZAPS II). The sampling techniques used were the same as those used at the Colstrip sites during 1974 and 1975 (Section 2).

Six samplings for each of the aboveground arthropods, soil macroarthropods, and microarthropods were taken in each of the two growing seasons, 1975 and 1976. Only the last four samplings for the soil macroarthropods were used in 1975 because of previously described sampling problems (see Colstrip section). Also, one intensified sampling was made for soil microarthropods in 1977. In that sampling, the number of samples per replicate was doubled to 10 per replicate from that in previous years samplings. An additional set of samples was taken beneath the pipe orifaces on the high concentration treatments on both ZAPS I and II.

The purpose of sampling the arthropod fauna was to attempt to detect major population or community-level changes in the fauna on the field plots as a result of exposure to the various SO_2 concentrations. These changes could be the result of direct toxicity of the SO_2, repellent effect of the SO_2, or indirect effects arising from changes in other ecosystem components. Emphasis here is placed on effects on the trophic structure of the arthropods. The data have not been statistically analyzed for significant population changes.

Aboveground Arthropods

Results of the sampling are presented for 1975 (Table 19.1) and 1976 (Table 19.2). The trophic groups do not show any indication of a treatment response with the possible exception of plant tissue feeders on ZAPS I in 1975. However, the same trend is lacking in 1976 on both ZAPS I and ZAPS II. The higher populations on ZAPS I in 1975 appear to be an artifact of the high variability of the data.

Soil Macroarthropods

Of the three arthropod groups censused on the ZAPS sites, the most apparent treatment responses were recorded among the soil macroarthropods. Although total numbers and biomass did not reflect major treatment response (Figure 19.1) definite population declines were noted for some constituent families and one trophic group. Most of the major trophic categories failed to show treatment responses (Tables 19.3 and 19.4); however, a sharp decline in the number of predators with increasing SO_2 concentration was noted for ZAPS I in 1975 and both ZAPS I and II in 1976, although the declines in ZAPS II were not as sharp as in ZAPS I. The decline in total predators was primarily a result of a similar decline in the largely predatory carabid beetles (Coleoptera-Carabidae) represented by *Harpalus desertus* (primarily), *Axinopalpus biplagiatus*, and *Bembidion nitidum* (Figure 19.2, Table 19.5). A

TABLE 19.1. ABOVEGROUND ARTHROPODS ON ZAPS I FOR 1975*

Sample date	Control	Low	Medium	High
		Unknown		
20 April	0.4	0.3	1.9	0.2
17 May	0.4	2.6	1.0	0.5
12 June	0.9	0.2	0.3	1.6
10 July	0.3	0.3	1.1	<0.1
4 August	6.0	0.6	2.6	1.3
17 September	0.0	0.0	<0.1	0.0
Mean	1.3	0.7	1.2	0.6
		Plant Tissue Feeders		
20 April	115.4	45.8	68.7	56.8
17 May	124.6	41.9	91.1	84.9
12 June	59.8	45.2	70.2	63.5
10 July	87.0	64.9	97.5	157.4
4 August	334.5	97.7	44.8	65.1
17 September	39.3	53.7	76.5	79.1
Mean	130.1	58.2	74.8	84.5
		Plant Sap Feeders		
20 April	1.9	4.5	6.8	11.4
17 May	2.8	4.8	5.8	5.2
12 June	37.3	43.2	57.1	59.7
10 July	30.7	23.0	22.5	36.2
4 August	16.1	7.6	10.0	16.9
17 September	23.3	9.4	3.6	3.0
Mean	18.7	15.4	17.6	22.1
		Plant Pollen Feeders		
20 April	3.1	1.5	1.9	1.3
17 May	0.7	0.2	0.6	0.6
12 June	1.6	0.6	1.0	0.5
10 July	9.2	16.2	12.8	16.2
4 August	6.8	5.8	0.1	0.0
17 September	1.7	0.6	1.2	1.6
Mean	3.8	4.2	2.9	3.4

TABLE 19.1. CONTINUED

Sample date	Control	Low	Medium	High
		Predators		
20 April	7.1	11.7	6.5	8.7
17 May	5.5	4.2	15.2	5.4
12 June	5.1	3.6	6.6	3.2
10 July	17.5	14.0	19.3	24.2
4 August	2.6	22.3	21.7	2.0
17 September	0.8	0.7	2.5	5.7
Mean	6.4	9.4	12.0	8.2
		Omnivores		
20 April	5.6	6.0	0.6	2.2
17 May	12.9	20.4	34.1	25.2
12 June	35.3	35.6	17.6	70.5
10 July	22.5	17.5	5.8	8.8
4 August	8.9	10.5	0.5	36.6
17 September	15.1	18.2	4.0	2.0
Mean	16.7	18.0	10.4	24.2
		Scavengers		
20 April	5.3	8.2	10.2	5.4
17 May	15.3	28.1	8.5	18.3
12 June	9.4	7.7	5.2	8.5
10 July	2.4	13.0	3.8	4.8
4 August	1.5	4.1	1.6	0.3
17 September	13.1	0.8	8.0	8.5
Mean	7.8	10.3	6.2	7.6
		Nonfeeding		
20 April	0.0	0.0	0.0	0.0
17 May	0.0	0.0	0.0	0.0
12 June	60.5	158.5	59.9	69.3
10 July	0.6	3.7	0.6	0.0
4 August	0.0	0.0	0.0	0.0
17 September	0.0	0.0	0.0	0.0
Mean	10.2	27.0	10.1	11.5

---

*Biomass (mg · m^{-2}) data are given by sample date and season mean.

TABLE 19.2. ABOVEGROUND ARTHROPODS ON ZAPS I AND ZAPS II FOR 1976*

Sample date	ZAPS I				Sample date	ZAPS II			
	Control	Low	Medium	High		Control	Low	Medium	High
				Plant Tissue Feeders					
22 March	53.5	59.4	70.4	110.5	24 March	142.4	117.4	124.7	80.3
17 May	45.1	36.6	32.4	22.4	21 May	51.2	73.4	44.5	27.4
15 June	52.7	37.8	43.2	63.7	20 June	33.2	61.7	55.5	32.2
10 July	44.1	53.6	48.1	48.1	14 July	48.9	53.3	79.4	62.1
9 August	62.3	45.1	77.7	49.6	13 August	74.8	87.4	109.1	45.2
6 September	41.2	66.5	25.9	71.9	11 September	93.2	69.2	63.2	88.3
Mean	49.8	49.9	49.6	61.0		74.0	77.1	79.4	55.9
				Plant Sap Feeders					
22 March	2.7	3.7	3.3	2.7	24 March	1.6	1.8	2.2	0.7
17 May	14.3	16.4	20.6	18.9	21 May	8.2	19.2	6.4	9.5
15 June	22.4	16.9	13.7	18.2	20 June	12.8	17.0	14.3	10.2
10 July	53.0	25.3	18.8	33.9	14 July	19.8	12.5	8.3	15.0
9 August	29.3	25.7	14.2	19.8	13 August	13.3	22.6	11.5	17.6
6 September	6.9	4.8	3.7	4.0	11 September	12.8	6.8	6.1	7.0
Mean	21.4	15.5	12.4	16.3		11.4	13.3	8.1	10.0
				Plant Pollen Feeders					
22 March	0.6	0.2	0.2	0.5	24 March	0.6	0.6	1.1	0.2
17 May	1.7	0.9	0.6	0.9	21 May	0.4	0.9	0.6	1.3
15 June	22.0	1.5	0.4	21.5	20 June	0.8	2.1	1.3	2.3
10 July	1.7	3.0	4.2	15.0	14 July	2.1	5.0	0.2	14.6
9 August	9.7	0.6	1.8	2.7	13 August	9.2	8.9	1.8	3.0
6 September	0.3	0.3	0.3	<0.1	11 September	0.6	0.2	1.2	<0.1
Mean	6.0	1.1	1.3	6.8		2.3	3.0	1.0	3.6

TABLE 19.2. CONTINUED

Sample date	ZAPS I Control	ZAPS I Low	ZAPS I Medium	ZAPS I High	Sample date	ZAPS II Control	ZAPS II Low	ZAPS II Medium	ZAPS II High
					Predators				
22 March	9.1	7.0	20.2	21.1	24 March	7.3	5.8	16.4	5.1
17 May	7.2	10.6	11.3	5.7	21 May	19.8	29.4	19.9	14.3
15 June	10.7	12.9	17.4	25.8	20 June	11.4	11.0	13.6	5.2
10 July	20.3	8.6	13.6	16.9	14 July	11.3	10.7	5.2	26.6
9 August	17.9	12.6	15.7	11.0	13 August	7.2	14.3	12.2	8.0
6 September	4.6	1.9	2.3	3.3	11 September	4.7	2.8	2.3	2.2
Mean	11.6	8.9	13.4	14.0		10.3	12.3	11.6	10.2
					Omnivores				
22 March	0.3	0.1	0.5	0.0	24 March	0.3	1.1	0.4	0.1
17 May	21.1	18.9	52.5	26.2	21 May	10.0	36.8	32.7	13.3
15 June	36.4	32.4	22.2	39.0	20 June	20.9	36.6	42.5	16.7
10 July	26.3	22.8	20.5	9.6	14 July	20.1	12.5	5.3	42.3
9 August	2.6	0.9	8.9	2.4	13 August	5.6	9.5	5.3	4.5
6 September	0.0	2.3	0.0	0.0	11 September	0.0	0.0	0.9	0.0
Mean	14.5	12.9	17.4	12.9		9.5	16.1	14.5	12.8
					Scavengers				
22 March	21.6	2.0	4.2	15.9	24 March	8.5	21.3	51.9	8.7
17 May	1.9	2.0	4.4	0.8	21 May	2.3	8.2	18.1	4.1
15 June	3.4	2.4	6.7	0.0	20 June	7.8	22.8	4.5	3.2
10 July	2.7	1.0	1.5	3.1	14 July	2.8	6.6	3.3	3.2
9 August	3.4	2.4	4.2	1.6	13 August	6.4	12.5	14.7	2.8
6 September	0.2	4.7	0.8	<0.1	11 September	2.8	8.5	3.7	7.0
Mean	5.5	2.4	3.6	3.6		5.1	13.3	16.0	4.8

[*] Biomass (mg · m^{-2}) data are given by sample date and season mean.

second beetle family, Curculionidae, though not predatory, also showed a decline in biomass with increased SO_2 exposure in both ZAPS I and II in 1976 (Table 19.6). The family was represented primarily by *Hyperodes vitticollis* and *H. grypidioides*; and secondarily by *Smicronyx* sp., *Apion* sp., and *Epicaerus*. A similar decline was noted in ZAPS I in 1975, i.e., 120, 50, 80, and 40 $mg \cdot m^{-2}$ for control, low, medium, and high treatments, respectively. The aforementioned declines in Carabidae and Curculionidae were responsible, in large part, for a decline in total Coleoptera biomass with increasing SO_2 exposure (Figure 19.2, Table 19.5).

In 1975, grasshopper (Orthoptera-Acrididae) egg biomass was substantially reduced in all SO_2 treated plots of ZAPS I (Figure 19.3). This was originally interpreted as indicative of decreased oviposition on the treated plots.

However, the 1976 data (not given) for both ZAPS I and II failed to substantiate this. Grasshopper population studies were intensified in 1976, and those results are detailed in the section entitled The Effect of Chronic SO_2 Air Pollution on Acrididae (Grasshoppers) Population Dynamics and Behavior.

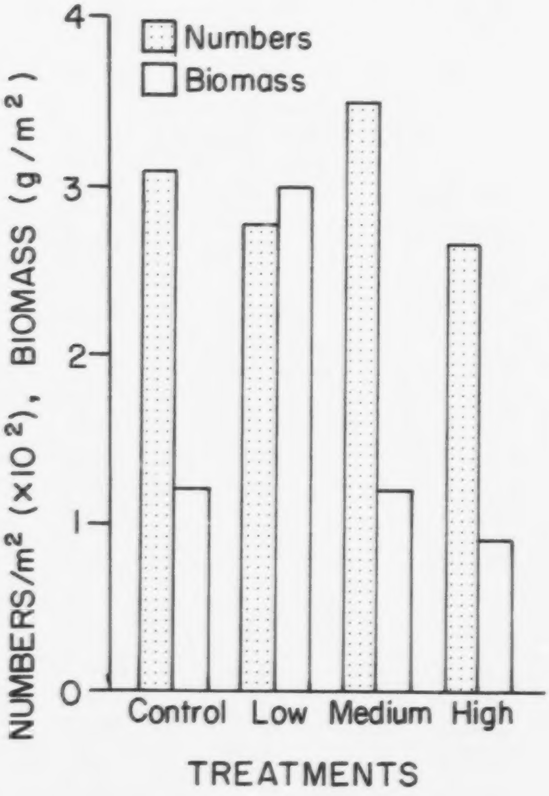


Figure 19.1. Total soil microarthropod numbers and biomass on four SO_2 fumigation treatments on the ZAPS I site for 1975 (based on time-weighted means of the four post treatment samplings).

TABLE 19.3. SOIL MACROARTHROPODS ON ZAPS I FOR 1975[*]

Sample date	Control	Low	Medium	High
		Root Tissue Feeders		
13 June	60.5	269.0	184.0	451.6
23 July	363.0	7,299.5	669.4	192.8
6 August	400.3	423.9	558.4	338.9
17 September	619.4	970.3	654.3	1,080.7
Mean	360.8	2,240.7	516.8	516.0
		Root Sap Feeders		
13 June	0.0	0.0	0.0	241.9
23 July	746.9	0.0	0.0	49.5
6 August	35.4	21.2	106.1	56.6
17 September	70.7	28.3	99.0	169.8
Mean	213.3	12.4	51.3	129.4
		Plant Tissue Feeders		
13 June	121.6	106.9	30.4	99.1
23 July	0.0	0.0	0.0	0.0
6 August	40.7	0.0	10.7	7.9
17 September	142.3	149.0	148.1	28.5
Mean	76.2	64.0	47.3	33.9
		Predators		
13 June	74.5	122.4	66.4	56.1
23 July	235.7	0.0	55.7	115.8
6 August	258.1	242.5	161.2	20.8
17 September	10.9	79.1	140.6	57.2
Mean	144.8	111.0	106.0	62.5
		Omnivores		
13 June	20.7	23.1	131.1	244.4
23 July	65.0	6.6	0.0	0.0
6 August	356.4	17.2	362.0	12.0
17 September	23.1	0.0	0.0	0.0
Mean	116.3	11.7	123.3	64.1

TABLE 19.3. CONTINUED

Sample date	Control	Low	Medium	High
		Nonfeeding		
13 June	301.1	201.5	331.4	246.6
23 July	423.5	380.1	741.9	263.1
6 August	68.9	633.1	348.8	58.8
17 September	0.0	97.4	0.0	114.2
Mean	198.9	328.0	355.5	170.7

*Biomass ($mg \cdot m^{-2}$) data are given by sample date and season mean.

One other macroarthropod group appeared to decline under SO_2 exposure in 1976. The plant-chewing *Crambus* sp. (Lepidoptera-Pyralidae) generally had lower populations in the treated areas in both ZAPS except for the high treatment of ZAPS I. The data for ZAPS II appear to be in strong support of the hypothesis that the SO_2 is causing population reductions in this and other arthropod groups.

The data for all the aforementioned groups include both adults and immatures. Therefore, the mechanism by which the SO_2 caused the population declines could be one or more of the following: (1) The SO_2 could act as a noxious repellent to the active adults, causing them to leave the treated areas and keeping others from entering. (2) In the case of predatory types, the prey items could be repelled as in (1). (3) Plant-feeding adults might be repelled by vegetation contaminated by deposited sulfur salts. (4) The SO_2 could be directly toxic to the insects. (5) The SO_2 could be indirectly detrimental to immature development through such mechanisms as reduced forage quality. Future studies will address these various possibilities.

Soil Microarthropods

From a qualitative evaluation of the data for both years and both ZAPS areas, no obvious treatment response has been observed for any trophic or taxonomic group although most all family-level data are yet to be evaluated. A slight treatment result was thought to be observed in the changing percent composition by major suborders of the total acarines on ZAPS I in 1975 (Figure 19.4). There was a decrease in Prostigmata with a corresponding increase in Cryptostigmata with increased SO_2 concentration. However, this trend was not observed for either ZAPS I or II in 1976 (Tables 19.7 and 19.8). There do not appear to be any major changes in intraseasonal dynamics of the soil microarthropods as exemplified in Figures 19.5 to 19.13. Further

TABLE 19.4. SOIL MACROARTHROPODS ON ZAPS I AND II FOR 1976*

Sample date	ZAPS I				Sample date	ZAPS II			
	Control	Low	Medium	High		Control	Low	Medium	High
Root Tissue Feeders									
21 March	0.0	137.7	344.9	1097.6	23 March	389.6	0.8	403.2	387.0
17 May	681.8	1062.4	634.3	1436.1	21 May	563.6	890.8	433.5	247.2
15 June	1030.6	468.9	1169.7	553.1	20 June	600.2	590.2	328.5	383.5
10 July	841.7	250.4	461.2	292.3	14 July	310.4	108.8	222.5	201.4
9 August	792.6	181.2	178.8	296.9	13 August	354.6	487.0	17.1	17.1
7 September	160.9	610.0	80.5	114.5	11 September	19.4	25.6	17.1	6.0
Mean	584.6	451.8	478.2	631.8		373.0	350.4	237.0	206.0
Root Sap Feeders									
21 March	0.0	0.0	0.0	0.0	23 March	0.0	0.0	0.0	0.0
17 May	0.0	0.0	0.0	0.5	21 May	0.0	0.0	0.0	0.0
15 June	0.0	0.0	0.0	0.0	20 June	0.0	0.0	0.0	0.0
10 July	28.3	0.0	42.4	70.7	14 July	0.0	99.0	113.2	0.0
9 August	0.0	0.0	0.0	725.7	18 August	141.5	84.9	70.7	28.3
7 September	84.9	0.0	0.0	0.0	11 September	0.0	0.0	0.0	0.0
Mean	18.9	0.0	7.1	132.8		23.6	30.6	30.6	4.7
Plant Tissue Feeders									
21 March	56.9	56.9	28.5	22.8	23 March	298.0	515.5	491.2	286.8
17 May	0.0	0.0	0.0	133.6	21 May	0.0	229.6	344.4	0.0
15 June	9.9	104.2	0.0	0.0	20 June	1052.0	358.6	344.4	0.0
10 July	0.0	229.6	0.0	124.1	14 July	574.0	803.6	344.4	230.0
9 August	125.5	550.7	56.3	116.9	13 August	1291.2	803.6	230.0	0.0
7 September	24.7	138.9	229.6	0.0	11 September	400.3	142.3	344.4	516.1
Mean	36.2	180.0	52.4	66.2		602.6	475.5	349.8	172.2

TABLE 19.4. CONTINUED

Sample date	ZAPS I Control	ZAPS I Low	ZAPS I Medium	ZAPS I High	Sample date	ZAPS II Control	ZAPS II Low	ZAPS II Medium	ZAPS II High
				Predators					
21 March	260.0	105.0	0.0	0.0	23 March	126.7	194.8	378.7	363.5
17 May	506.7	316.7	41.6	0.0	21 May	1244.9	484.9	273.2	443.3
15 June	633.3	268.9	0.0	0.0	20 June	380.0	190.0	321.1	253.3
10 July	263.1	126.8	380.0	41.6	14 July	570.0	506.7	168.3	743.1
9 August	83.2	4.8	124.9	0.0	13 August	778.1	190.0	273.2	253.3
7 September	483.2	166.5	334.8	41.6	11 September	503.1	336.6	126.7	63.3
Mean	371.7	164.8	146.9	13.9		600.5	317.2	256.9	353.3
				Omnivores					
21 March	0.0	551.7	702.4	0.0	23 March	0.0	0.0	0.0	0.0
17 May	12.8	0.0	1592.8	0.0	21 May	0.0	244.1	0.0	0.0
15 June	32.9	239.6	164.9	9.5	20 June	120.8	3.2	0.0	9.5
10 July	1310.2	50.7	22.0	41.2	14 July	18.7	98.8	117.2	0.0
9 August	3.1	0.0	0.0	0.0	13 August	0.0	0.0	0.0	0.0
7 September	0.0	0.0	0.0	0.0	11 September	0.0	0.0	0.0	0.0
Mean	226.5	140.3	413.7	8.45		23.2	57.7	19.5	1.6
				Scavengers					
21 March	0.0	69.1	0.0	0.0	23 March	69.1	138.2	169.5	276.4
17 May	69.1	138.2	236.3	0.0	21 May	36.2	145.4	14.5	0.0
15 June	152.7	0.0	0.0	0.0	20 June	7.2	83.6	0.0	0.0
10 July	149.9	0.0	5.8	29.3	14 July	51.6	0.0	5.8	7.2
9 August	0.0	0.0	0.0	0.0	13 August	0.0	17.6	0.0	5.8
7 September	0.0	0.0	0.0	0.0	11 September	0.0	0.0	0.0	0.0
Mean	62.0	34.6	40.4	4.9		27.4	64.1	31.6	48.2

*Biomass (mg · m^{-2}) data are given by sample date and season mean.

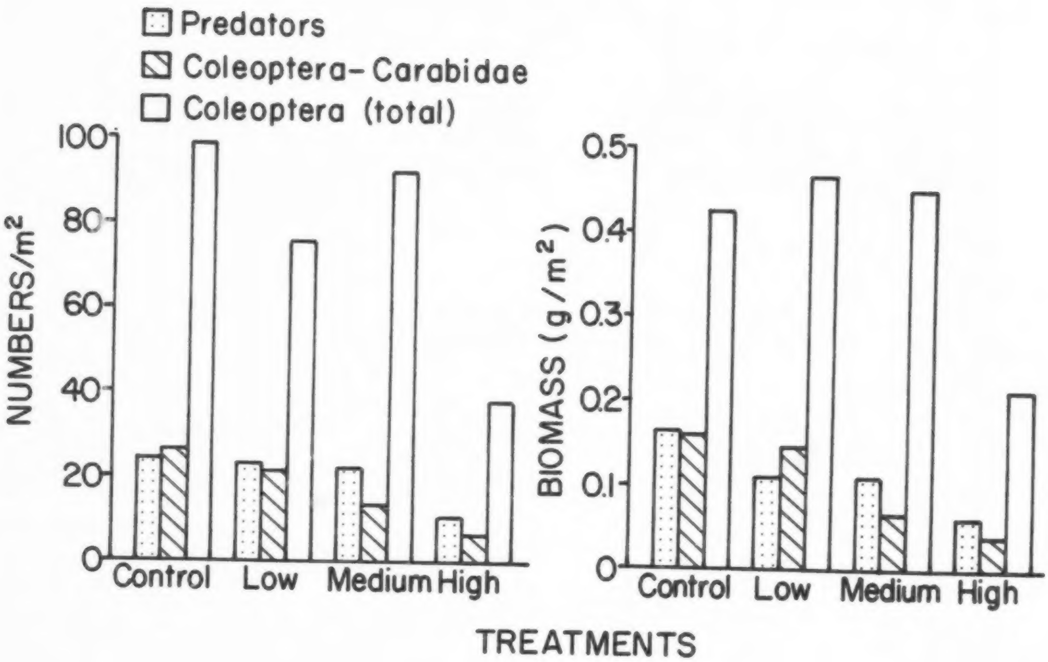


Figure 19.2. Numbers and biomass of predators, Coleoptera-Carabidae, and total Coleoptera on four SO_2 fumigation treatments on the ZAPS I site for 1975 (based on time-weighted means of the four post treatment samplings).

TABLE 19.5. BIOMASS (MG · M^{-2}) OF TOTAL COLEOPTERA AND FAMILY CARABIDAE (COLEOPTERA) FOR 1976

Sample date	ZAPS I				Sample date	ZAPS II			
	Control	Low	Medium	High		Control	Low	Medium	High
				Order Coleoptera					
21 March	316.4	258.0	95.4	153.7	23 March	424.6	710.3	1052.4	603.5
17 May	506.7	492.0	85.4	446.3	21 May	1336.0	687.1	967.9	680.2
15 June	1176.9	534.3	1096.8	45.4	20 June	1816.7	749.2	899.4	636.8
10 July	526.6	446.8	497.8	138.3	14 July	1423.7	419.0	621.1	1111.6
9 August	138.9	618.5	64.8	125.4	13 August	1861.2	993.6	436.7	270.4
7 September	384.1	164.5	364.8	17.1	11 September	673.1	421.2	488.1	579.4
Mean	508.3	419.0	367.5	154.4		1255.9	830.1	744.3	647.0
				Family Carabidae					
21 March	259.5	63.3	--	--	23 March	241.5	654.0	780.6	546.3
17 May	506.7	316.7	--	114.8	21 May	1203.3	672.9	534.4	443.3
15 June	633.3	253.3	--	--	20 June	1413.2	534.4	694.9	253.3
10 July	194.8	356.3	380.0	114.8	14 July	1144.0	1310.2	471.1	931.1
9 August	--	464.0	--	--	13 August	1832.8	993.6	419.6	253.3
7 September	316.7	--	356.3	--	11 September	482.9	253.3	471.1	522.5
Mean	318.5	242.3	122.7	38.3		1053.0	736.4	562.0	491.6

TABLE 19.6. BIOMASS (MG · M^{-2}) DATA FOR COLEOPTERA AND LEPIDOPTERA, ZAPS I AND II IN 1976

Sample date	ZAPS I				Sample date	ZAPS II			
	Control	Low	Medium	High		Control	Low	Medium	High
Order Coleoptera (Family Curculionidae)									
21 March	56.9	56.9	28.5	15.9	23 March	--	56.3	--	25.2
17 May	--	--	--	18.8	21 May	--	--	--	--
15 June	31.2	15.6	--	31.2	20 June	80.9	186.3	62.1	46.7
10 July	248.5	62.1	46.7	--	14 July	248.5	108.8	93.2	62.1
9 August	90.2	88.6	28.5	113.8	13 August	28.5	--	--	--
7 September	--	28.5	--	--	11 September	170.7	142.3	--	56.9
Mean	71.1	42.0	17.3	30.0		88.1	82.3	25.9	31.8
Order Lepidoptera (Family Pyralidae)									
21 March	584.4	389.6	194.8	1168.8	23 March	389.6	39.5	194.8	487.0
17 May	681.8	876.6	487.0	1071.4	21 May	389.6	876.6	--	--
15 June	487.0	292.2	--	487.0	20 June	194.8	389.6	97.4	--
10 July	584.4	97.4	--	194.8	14 July	--	--	--	--
9 August	779.2	97.4	97.4	194.8	13 August	292.2	487.0	--	--
7 September	97.4	584.4	--	97.4	11 September	--	--	--	--
Mean	535.7	389.6	129.9	535.7		211.0	298.8	48.7	81.2

data analyses, including statistical analyses, may reveal some treatment responses not yet noted.

The data for 1977 again fail to show substantial population changes in either ZAPS system (Tables 19.9 and 19.10). However, in both plots, the highest total populations were observed in the medium concentration plots of both ZAPS with the low in ZAPS I and low and high concentrations in ZAPS II showing populations higher than the controls. The populations in the extra high treated areas were at or below control levels. It is difficult to ascribe these changes to SO_2 exposure since the general acarine population (which makes up 90% and over of the total) remains proportionately the same for each suborder with the possible exception of ZAPS I where the percent of Prostigmata declined in the high and extra high treatments along with increases in the Mesostigmata and Cryptostigmata. The trophic structure is essentially constant across the treatments. The proportionate structure (by suborder) of the acarines is also the same as those given for 1976 (Tables 19.7 and 19.8). The soil microarthropods are definitely responding very slowly, if at all, to the SO_2 treatments.

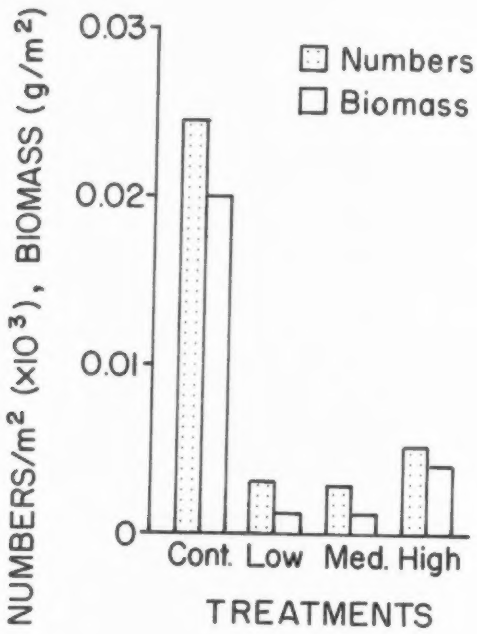


Figure 19.3. Numbers and biomass of grasshopper eggs on four SO_2 fumigation treatments on the ZAPS I site for 1975 (based on time-weighted means of the four post treatment samplings).

THE EFFECT OF CHRONIC SO_2 AIR POLLUTION ON ACRIDIDAE (GRASSHOPPERS) POPULATION DYNAMICS AND BEHAVIOR

During the 1977 field season, grasshopper reactions to the SO_2 fumigation on the ZAPS site were observed in three separate experiments. The primary experiment was a censusing of the grasshoppers on each treatment. The second experiment, or feeding preference test, was performed to determine the effect of sulfur compounds in and on plants exposed to SO_2 on feeding by selected species of grasshoppers. A third experiment was conducted to determine the effect of SO_2 on fecundity and survival in *Aulocara elliotti* (Thomas) (big-headed grasshopper) by establishing caged test populations on the control and high treatments of ZAPS I. The latter two experiments were designed to help explain treatment effects observed in the grasshopper census.

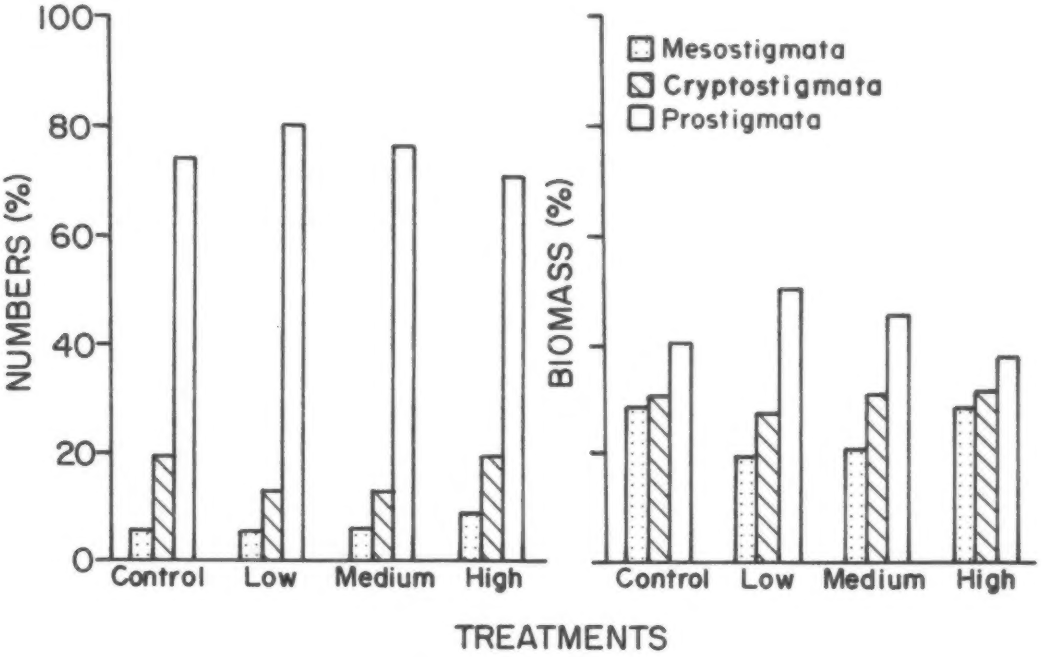


Figure 19.4. Composition, by suborders of total soil Acarina using numbers and biomass on four SO_2 fumigation treatments on ZAPS I site in 1975 (based on season time-weighted means). Total numbers of soil Acarina/m^2 for control, low, medium, and high treatments were 86,081.5, 82,194.8, 73,315.5, and 73,254.3, respectively. Total biomass for control, low, medium, and high treatments were 36.0, 28.7, 27.5, and 33.6 mg · m^{-2}, respectively.

TABLE 19.7. TIME WEIGHTED SEASONAL MEANS AND PERCENT COMPOSITION FOR SOIL MICROARTHROPODS*

Group	Season mean (number · m^{-2}) A	B	C	D	Season mean ($mg \cdot m^{-2}$) A	B	C	D
Total Acarina	112,329	119,482	155,239	123,568	46.50	38.04	49.25	42.69
Astigmata	515	908	1,454	698	0.39	0.68	1.09	0.47
Cryptostigmata	18,047	11,736	20,974	11,784	12.55	5.80	12.36	6.63
Prostigmata	87,900	100,875	126,378	104,358	19.83	21.88	27.17	22.08
Mesostigmata	5,867	5,963	6,433	6,728	13.73	9.68	8.63	13.51
Total microarthropods	144,557	139,476	177,914	144,341	81.73	62.81	78.15	68.01
Acarina (% of total)	77.7	85.7	87.2	85.6	56.9	60.6	63.0	62.8
% Astigmata[†]	0.4	0.8	0.9	0.6	0.8	1.8	2.2	1.1
% Cryptostigmata	16.1	9.8	13.5	9.5	27.0	15.2	25.1	15.5
% Prostigmata	79.2	84.4	81.4	84.4	42.6	57.5	55.2	51.7
% Mesostigmata	5.2	5.0	4.1	5.4	29.5	25.4	17.5	31.6
Trophic groups								
Fungivores	57,809	39,600	55,933	49,822	42.46	23.59	32.05	26.73
Unknown	33,656	27,322	32,725	45,655	577.91	400.32	459.24	1096.78
Plant sap feeders	39,939	54,668	70,334	42,085	10.54	10.15	15.19	9.63
Predators	19,176	22,111	23,569	18,543	22.50	24.22	24.47	24.32
Scavengers	304	571	313	195	0.01	0.02	0.01	0.01
Nonfeeding stage	24	39	71	146	0.01	0.01	0.02	0.04

*ZAPS I, 1976 (A = Control, B = Low, C = Medium, D = High).

[†] Suborders are % of total Acarina.

TABLE 19.8. TIME WEIGHTED SEASONAL MEANS AND PERCENT COMPOSITION FOR SOIL MICROARTHROPODS*

Group	Season mean (number · m^{-2})				Season mean (mg · m^{-2})			
	A	B	C	D	A	B	C	D
Total Acarina	118,256	103,657	155,583	129,200	44.96	38.18	54.27	45.98
Astigmata	1,428	589	1,207	1,367	1.08	0.45	0.93	1.03
Cryptostigmata	16,988	5,866	25,168	21,318	14.09	9.30	18.72	16.26
Prostigmata	94,605	81,749	122,800	101,211	21.04	17.97	26.02	22.01
Mesostigmata	5,235	5,453	6,408	5,304	8.75	10.46	8.60	6.68
Total microarthropods	143,998	134,401	182,217	160,908	70.69	69.26	82.98	83.77
Acarina (% of total)	82.1	77.1	85.4	80.3	63.6	55.1	65.4	54.9
% Astigmata†	1.2	0.6	0.8	1.0	2.4	1.2	1.7	2.2
% Cryptostigmata	14.4	15.3	16.2	16.5	31.3	24.4	35.5	35.4
% Prostigmata	80.0	78.9	78.9	78.3	46.8	47.1	47.9	47.9
% Mesostigmata	4.4	5.3	4.1	4.1	19.5	27.4	15.8	14.5
Trophic groups								
Fungivores	56,942	57,995	73,462	63,593	37.33	35.58	43.69	40.90
Unknown	15,058	12,758	15,854	13,465	344.38	322.86	484.50	644.72
Plant sap feeders	55,806	47,485	73,370	70,543	12.86	12.58	15.92	22.58
Predators	19,733	19,052	24,279	20,035	17.31	18.74	20.63	18.12
Scavengers	200	654	590	357	0.01	0.03	0.02	0.01
Nonfeeding stage	48	18	14	53	0.01	0.01	>0.00	0.02

*ZAPS II, 1976 (A = Control, B = Low, C = Medium, D = High).

†Suborders are % of total Acarina.

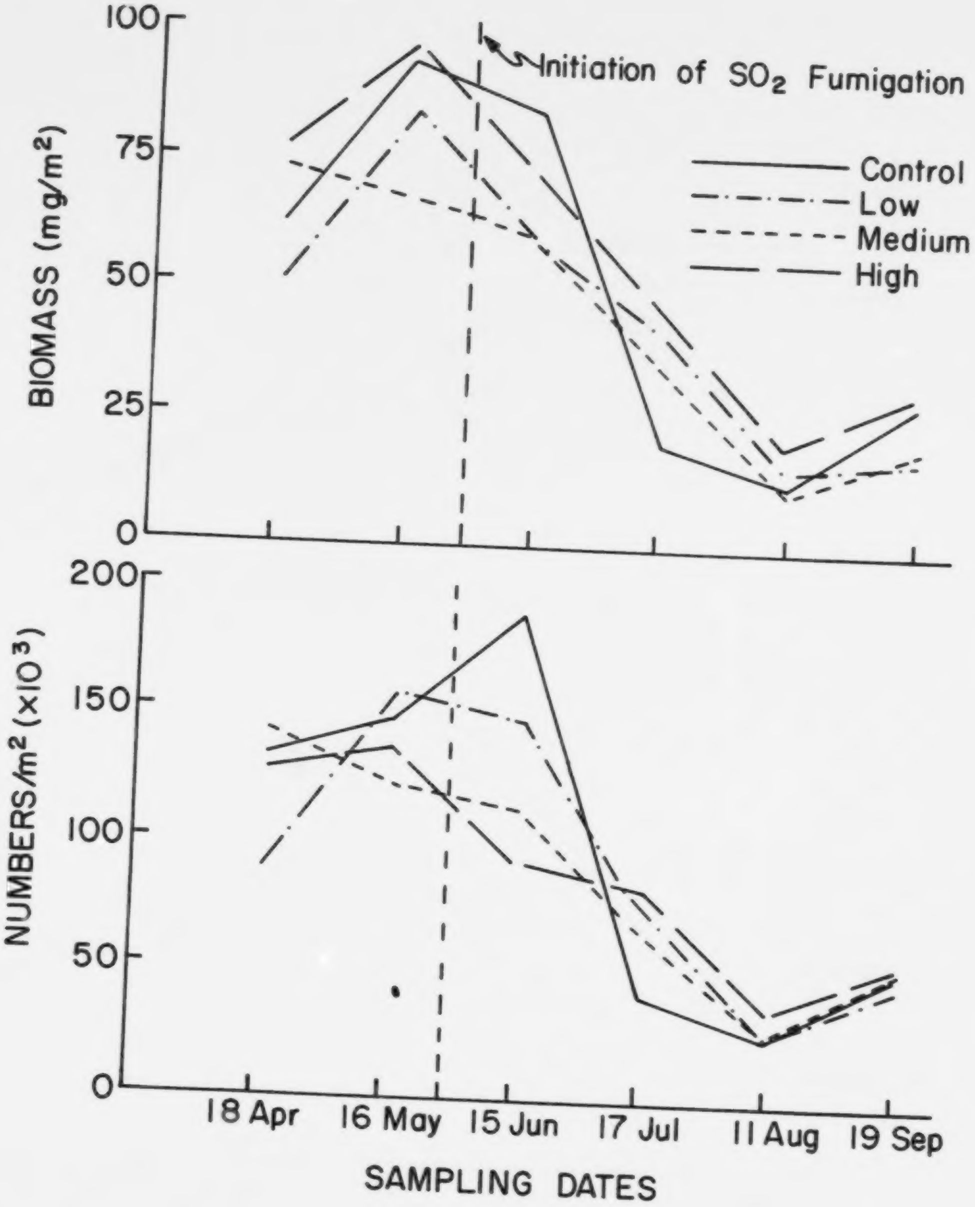


Figure 19.5. Total soil microarthropod biomass and numbers on four SO_2 fumigation treatments on the ZAPS I site for 1975 (six sample dates).

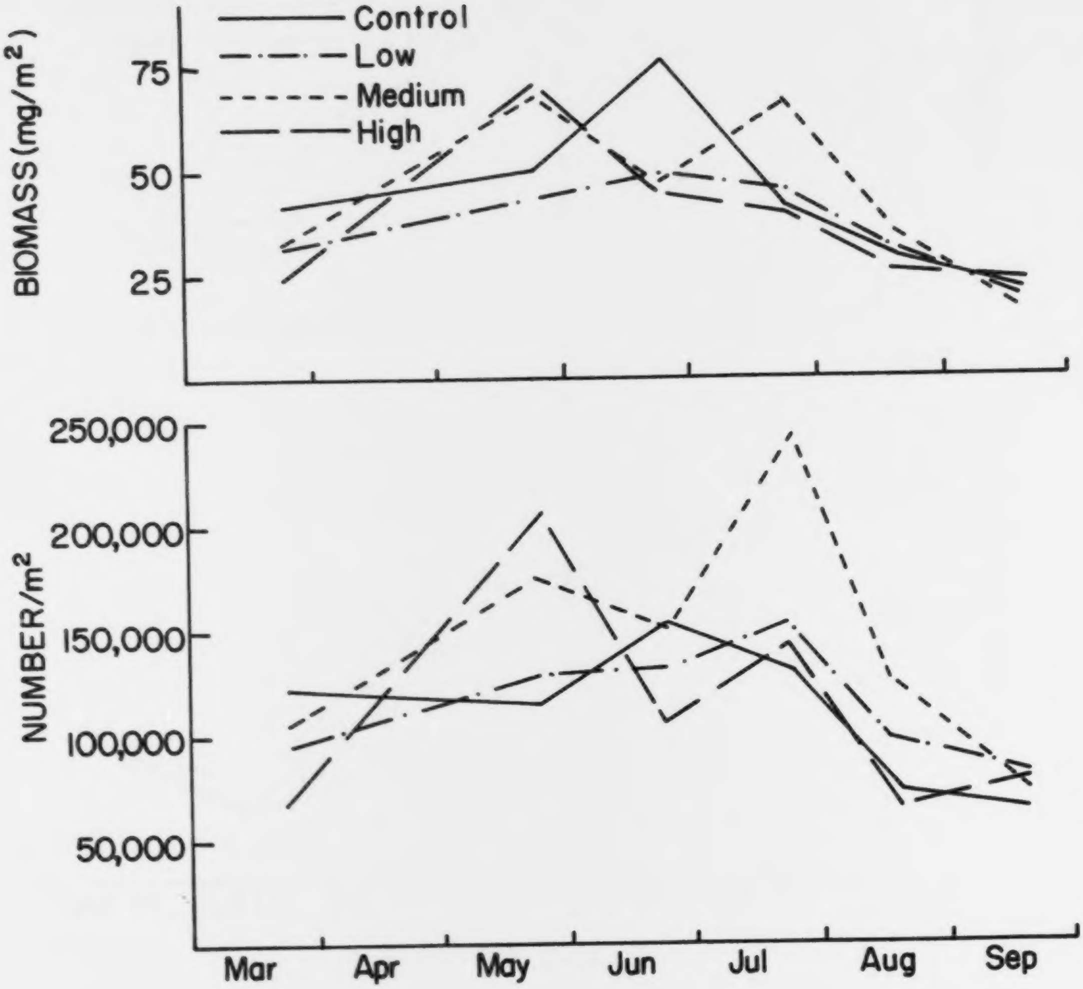


Figure 19.6. Seasonal dynamics of soil Acarina on ZAPS I, 1976.

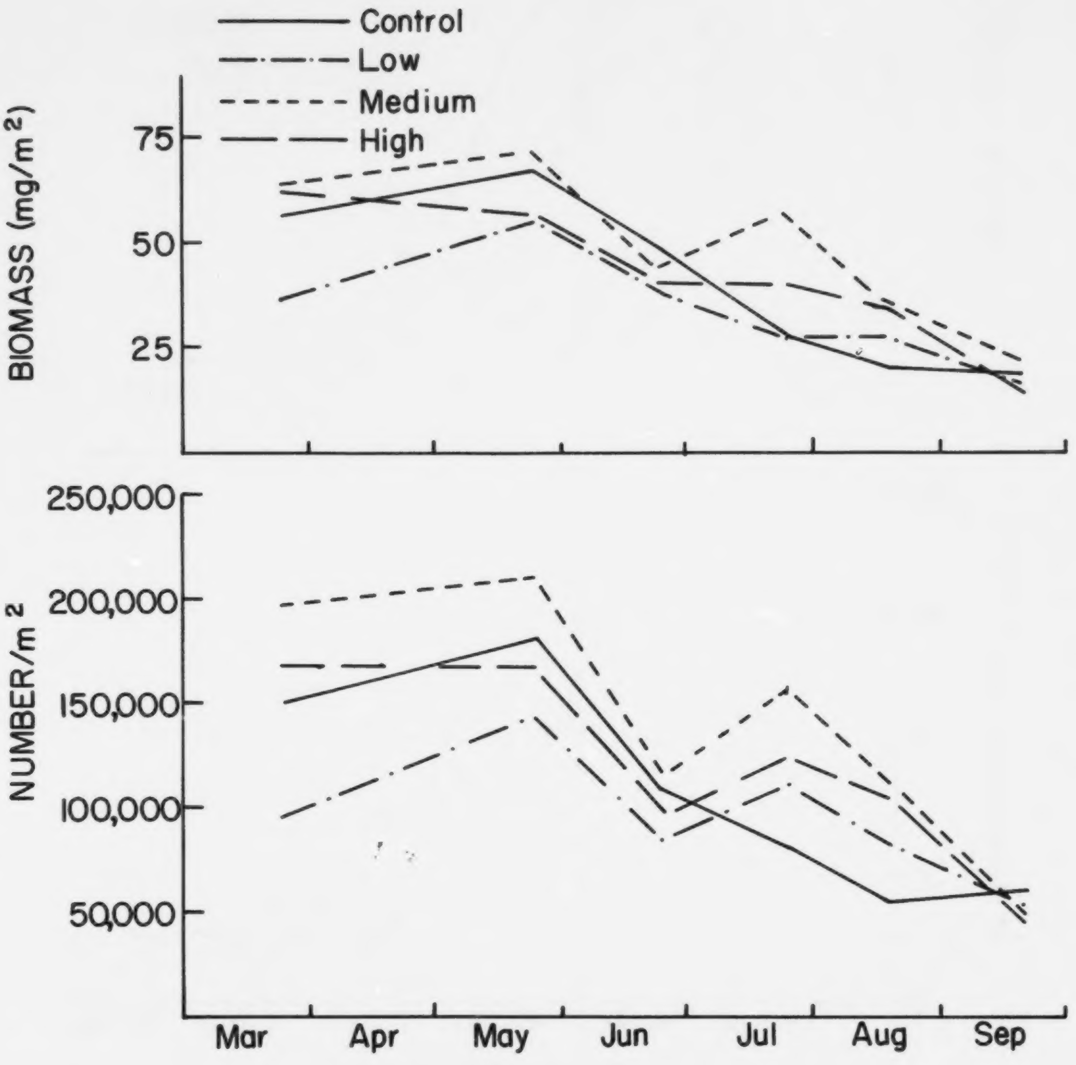


Figure 19.7. Seasonal dynamics of soil Acarina on ZAPS II, 1976.

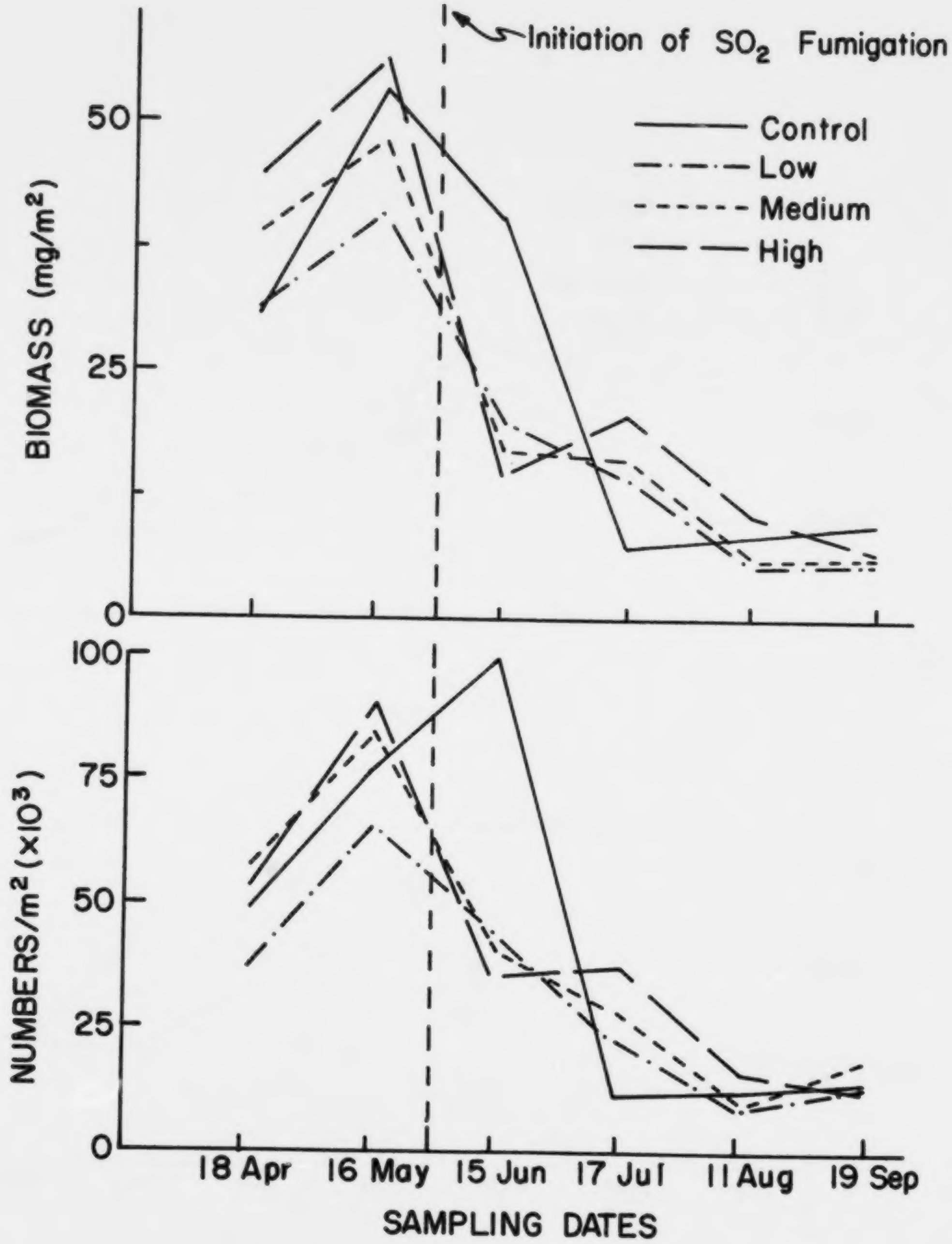


Figure 19.8. Biomass and numbers of the soil microarthropod trophic group, fungivore, on four SO_2 fumigation treatments on the ZAPS I site for 1975 (six sample dates).

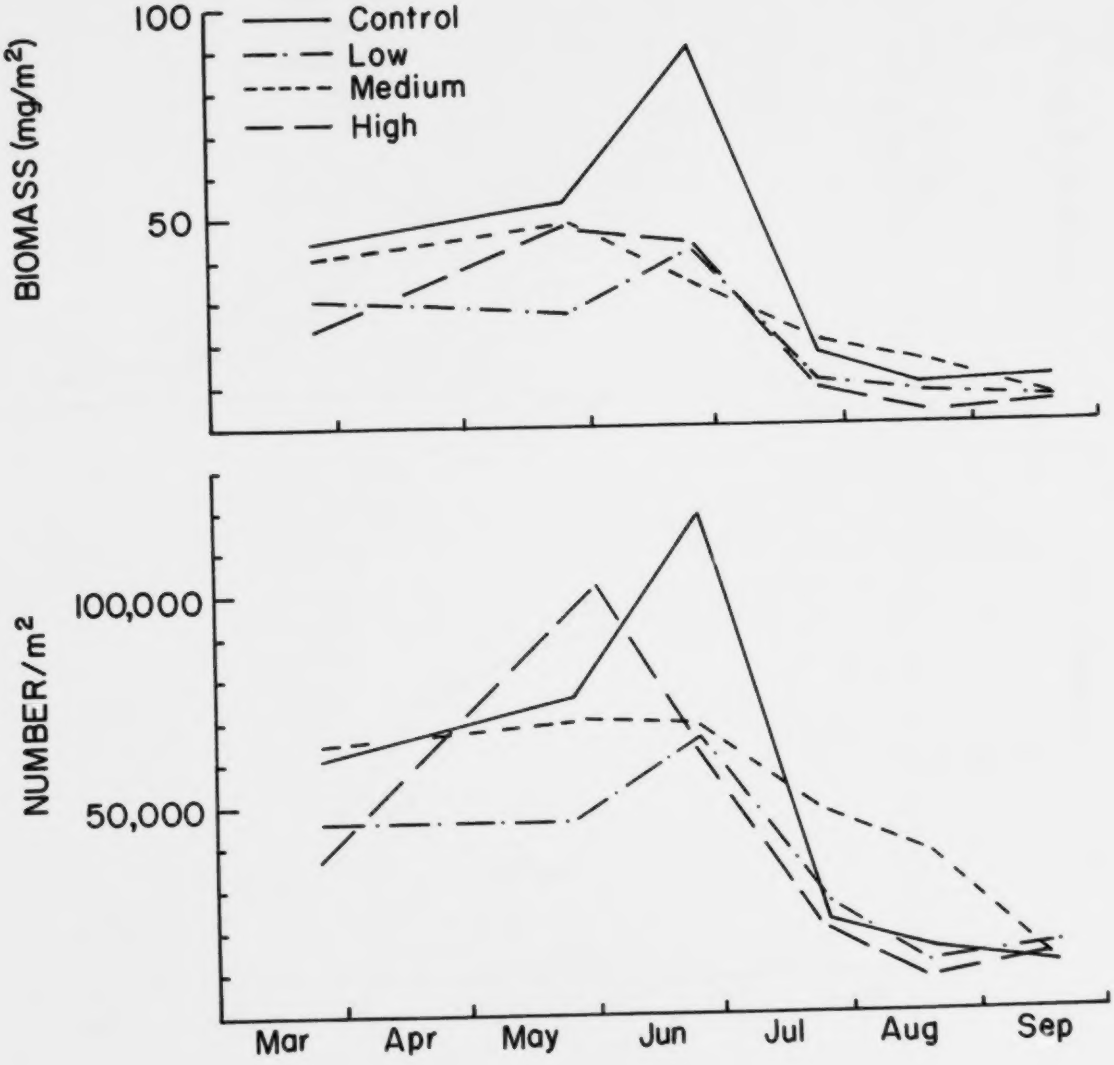


Figure 19.9. Seasonal dynamics of soil microarthropod fungivores on ZAPS I, 1976.

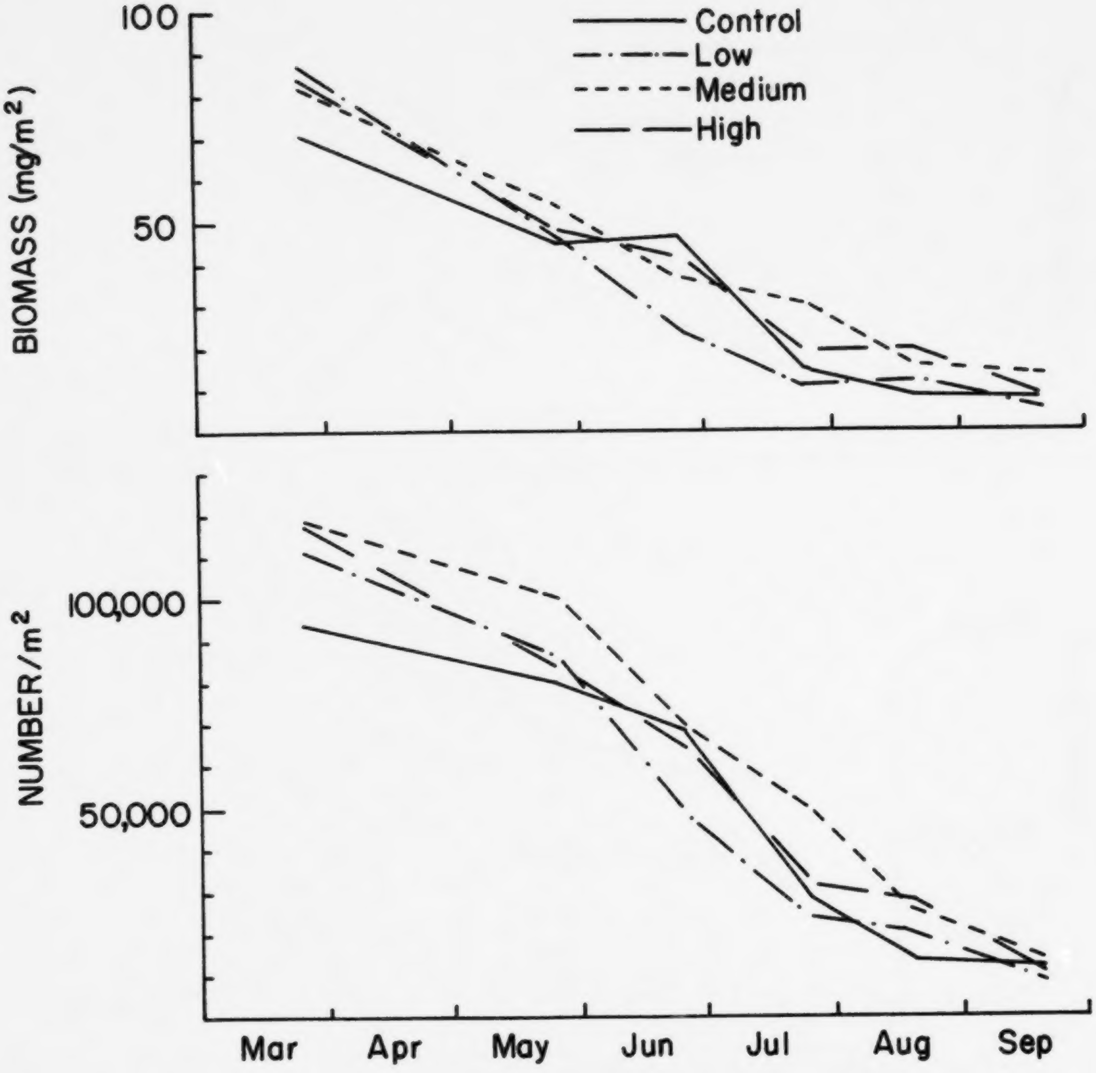


Figure 19.10. Seasonal dynamics of soil microarthropod fungivores on ZAPS II, 1976.

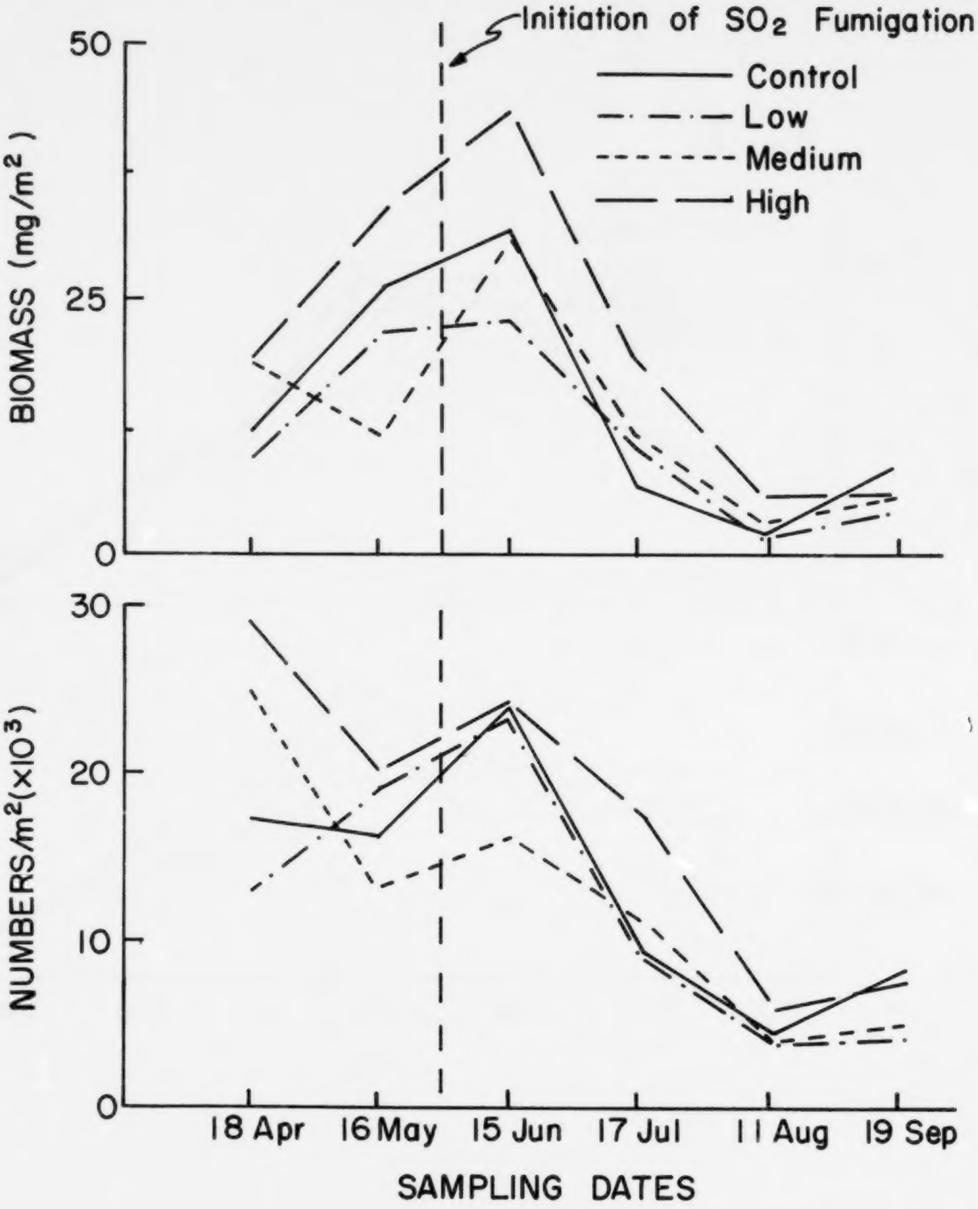


Figure 19.11. Biomass and numbers of the soil microarthropod trophic group, predator, on four SO_2 fumigation treatments on the ZAPS I site in 1975 (six sample dates).

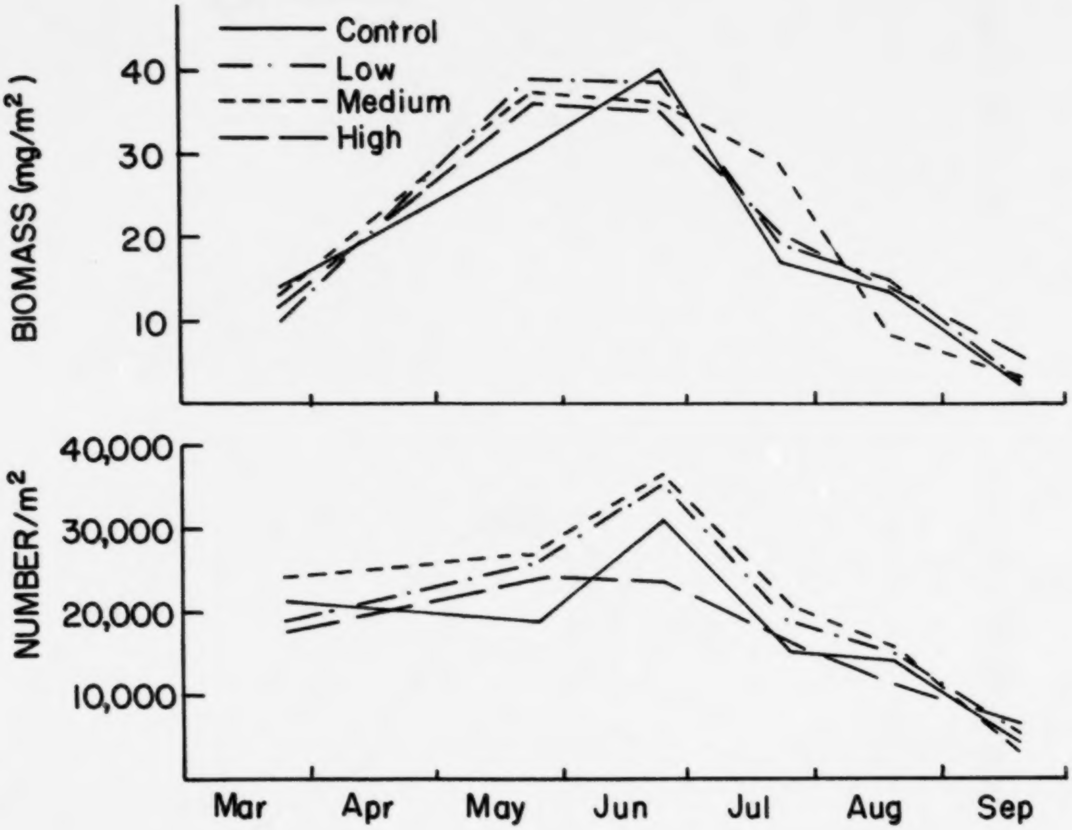


Figure 19.12. Seasonal dynamics of soil microarthropod predators on ZAPS I, 1976.

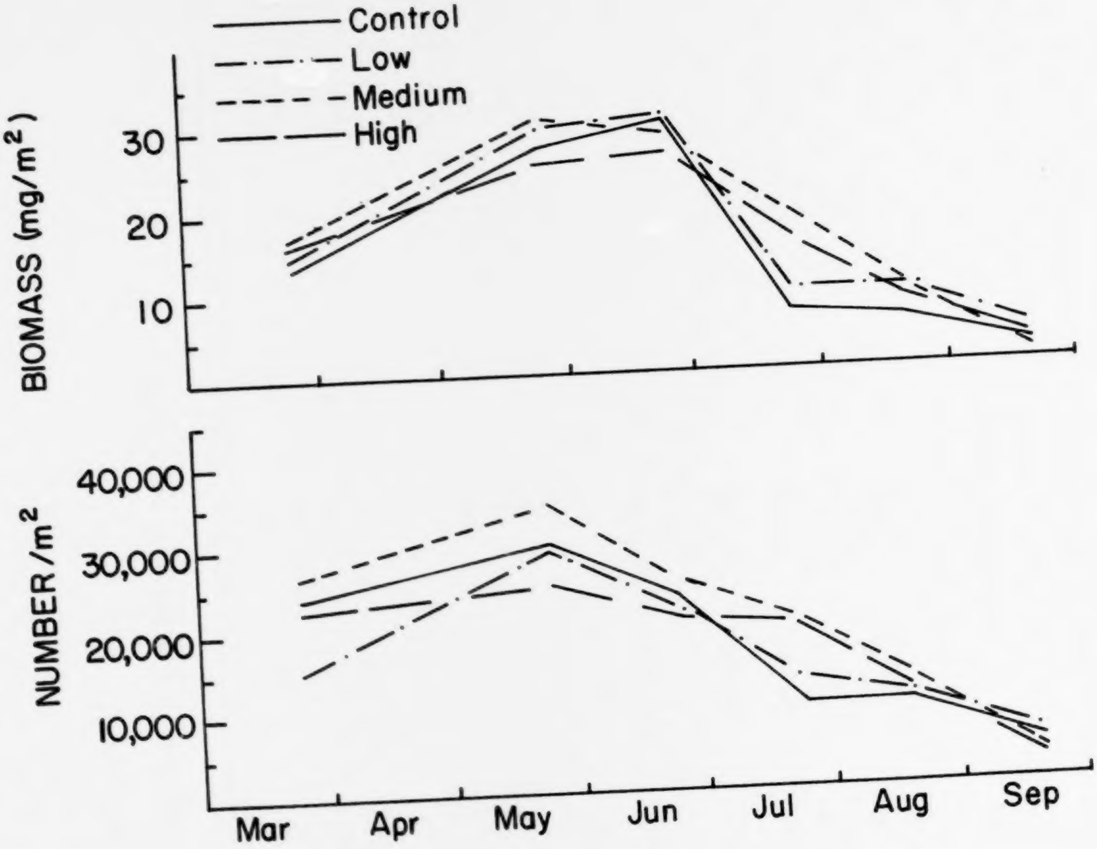


Figure 19.13. Seasonal dynamics of soil microarthropods on ZAPS II, 1976.

TABLE 19.9. TAXONOMIC AND TROPHIC STRUCTURE OF THE SOIL MICROARTHROPODS ON ZAPS I ON 10 JULY 1977*

Group	Numbers · m^{-2}					Mg · m^{-2}				
	A	B	C	D	E	A	B	C	D	E
Total Acarina	119,615	140,090	177,033	89,939	122,406	46.5	44.7	64.9	42.1	55.8
Astigmata	995	2,432	2,017	276	1,326	0.8	1.9	1.5	0.2	0.6
Cryptostigmata	13,705	15,501	23,873	13,042	20,530	9.9	7.4	16.8	10.4	14.1
Prostigmata	99,665	116,272	143,018	68,525	87,038	21.6	22.8	31.3	16.2	20.4
Mesostigmata	5,250	5,885	8,124	8,096	13,512	14.2	12.7	15.3	15.2	20.7
Collembola	2,321	2,404	2,653	2,432	3,813	2.2	2.0	1.8	2.3	3.0
Total microarthropods	124,008	145,091	182,504	94,111	128,733	52.0	50.7	71.0	46.5	61.0
Acarina (% of total)	96.0	96.6	97.0	95.6	95.1	89.4	88.2	91.4	90.5	91.5
% Astigmata†	0.8	1.7	1.1	0.3	1.1	1.7	4.3	2.3	0.5	1.1
% Cryptostigmata	11.5	11.1	13.5	14.5	16.8	21.3	16.6	25.9	24.7	25.3
% Prostigmata	83.3	83.0	80.8	76.2	71.1	46.6	51.0	48.2	38.5	36.6
% Mesostigmata	4.4	4.2	4.6	9.0	11.0	30.5	28.4	23.6	36.1	37.1
Trophic groups										
Fungivores	22,492	24,509	32,080	22,713	29,455	14.0	12.0	20.8	14.2	18.3
Herbivores	71,951	93,061	119,394	43,022	57,721	14.2	18.0	24.4	8.7	10.1
Predators	22,132	17,712	24,094	22,713	30,560	21.7	18.9	24.0	21.9	29.5
Scavengers	28	28	83	166	884	<0.0	<0.0	<0.0	<0.0	<0.0

*A = Control, B = Low, C = Medium, D = High, E = Extra High.

†Suborders are % of total Acarina.

TABLE 19.10. TAXONOMIC AND TROPHIC STRUCTURE OF THE SOIL MICROARTHROPODS ON ZAPS II ON 7 AUGUST 1977[*]

Group	Numbers · m^{-2}					Mg · m^{-2}				
	A	B	C	D	E	A	B	C	D	E
Total Acarina	95,907	128,015	170,594	148,710	80,931	31.4	46.6	63.5	55.8	26.9
Astigmata	304	414	332	1,050	442	0.2	0.3	0.3	0.8	0.3
Cryptostigmata	10,555	16,192	20,585	20,309	13,069	8.9	14.4	17.0	16.4	7.9
Prostigmata	80,738	105,523	141,112	118,952	63,828	17.5	22.5	28.0	26.1	14.1
Mesostigmata	4,310	5,885	8,566	8,400	3,592	4.9	9.3	18.2	12.6	4.6
Collembola	1,768	3,067	7,571	11,495	13,788	1.6	2.3	5.5	10.5	8.7
Total microarthropods	101,738	133,844	203,309	163,824	97,152	39.1	53.3	108.5	72.6	41.5
Acarina (% of total)	94.3	95.6	83.9	90.8	83.3	80.3	87.3	58.5	76.9	64.8
% Astigmata[†]	0.3	0.3	0.2	0.7	0.6	0.6	0.6	0.5	1.4	1.1
% Cryptostigmata	11.0	12.7	12.1	13.7	16.2	28.3	30.9	26.8	29.4	29.4
% Prostigmata	84.2	82.4	82.7	80.0	78.9	55.7	48.3	44.1	46.8	52.4
% Mesostigmata	4.5	4.6	5.0	5.7	4.4	15.6	20.0	28.7	22.6	17.1
Trophic groups										
Fungivores	22,961	25,200	37,247	37,689	31,831	12.8	18.2	24.7	28.6	17.8
Herbivores	57,970	79,108	138,707	94,830	45,453	14.3	15.9	57.2	20.5	8.5
Predators	13,650	15,888	22,796	24,371	15,280	8.9	14.0	24.6	20.2	13.3
Scavengers	28	--	--	--	332	<0.0	--	--	--	0.01

[*] A = Control, B = Low, C = Medium, D = High, E = Extra High.

[†] Suborders are % of total Acarina.

Grasshopper Census

A census of the grasshopper populations was made on each treatment of both ZAPS sites, starting in early May and continuing through August. The census method used was one employed by the USDA-Plant Protection and Quarantine Division to census outbreaks and make control recommendations for the grasslands of eastern Colorado. This census method involved walking a predetermined transect (see Figure 19.14) on each replicate and along this line, 25 one-ft^2 areas were observed for grasshoppers. Most identifications were made to the species level with estimate of the instars observed. These data have been converted to number per m^2 by age of species (instar), species, sub-family, and total count. The censuses were made in the morning, usually between 9:00 a.m. and noon, on clear days when the wind was not excessive. Censusing was done before other investigators had disturbed the treatments. A census was made eight times on each ZAPS sites. The census dates were:

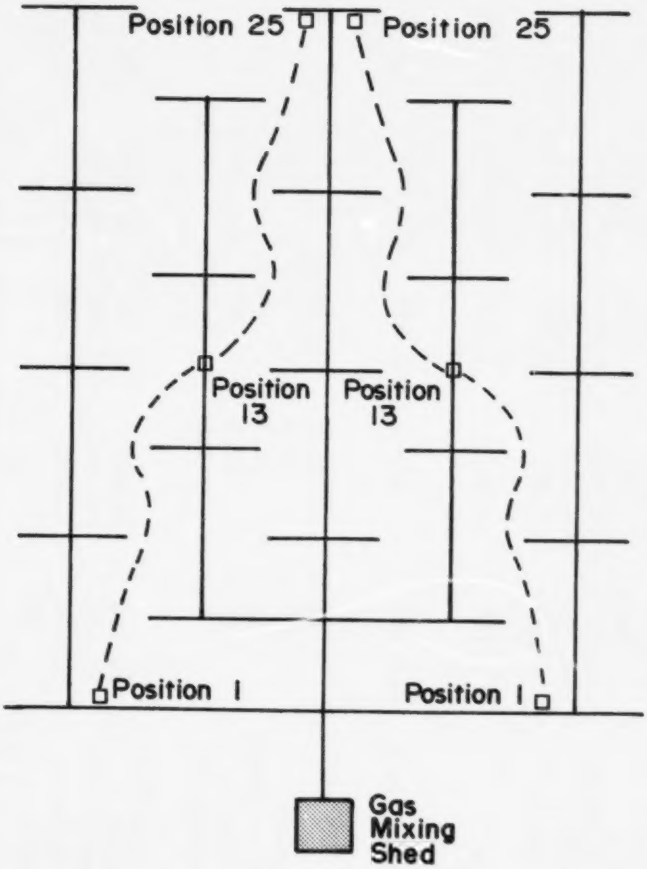


Figure 19.14. Route of transect used for the grasshopper census within a given treatment plot.

ZAP I	ZAP II
10 May 1977	10 May 1977
24 May 1977	24 May 1977
7 June 1977	7 June 1977
15 June 1977	15 June 1977
24 June 1977	24 June 1977
7 July 1977	7 July 1977
22 July 1977	22 July 1977
3 August 1977	12 August 1977

Melanoplus sanquinipes (Fab) was by far the most common grasshopper observed. See Table 19.11 for the relative numbers of grasshoppers counted in the census. Other species observed on the ZAPS sites that were not included in the counts were *M. bivitattus* (Say), *Dissosteria carolina* (Linnaeus), *Metator pardalinus* (Saussare), and *Spharagemon collare* (Scudder).

Statistical analysis has not yet been performed on the data, but the trend is for decreasing numbers of grasshoppers with increasing SO_2 concentrations (see Figure 19.15). Because the sample data have a non-normal distribution, they were pooled by replicate and the mean and standard error for a treatment is based on a sample size of two. The greatest differences between treatments were in the latter half of the season so it was thought that this might be due to the adults (more mobile than nymphs) leaving the higher concentration treatments, thereby decreasing the mean instar of the remaining population. This was not observed (Figure 19.16). Thus, the differences between treatments were either present before the beginning of the 1977 season or are the result of population decreases independent of age of grasshoppers. Most likely the later season differences occur because of differential oviposition during the previous years of fumigation as these differences do not appear until the time of hatching. This is supported by biomass sampling of grasshopper eggs in 1975 (Dodd *et al.*, 1977). Although data are not yet compiled from the survival study, they should provide further evidence for the support or rejection of this hypothesis. A second hypothesis for explaining the trend toward fewer grasshoppers with increased SO_2 is emigration from the fumigated treatments because of a decrease in food quality or selectivity against SO_2-exposed food. The feeding preference experiment lends some support to this.

Feeding Preference Test

This experiment was designed to test for an effect of chemical derivatives of SO_2 deposited in and on plants on the feeding of grasshoppers. There have been many studies on the food preference of grasshoppers, most dealing with determining a preference for different species of plant as food. A few have dealt with the influence of plant chemicals on food selection. Thorsteinson and Nayar (1963) found that certain phospholipids in wheat stimulated feeding by grasshoppers. In a later study, Harley and Thorsteinson (1967) tested the reaction of *Melanoplus bivittatus* to 20 secondary plant chemicals. They found that the rejection of certain plant chemicals may have been due to the chemical's form, with most salts being rejected. This is important to this experiment because SO_2 is dissolved in

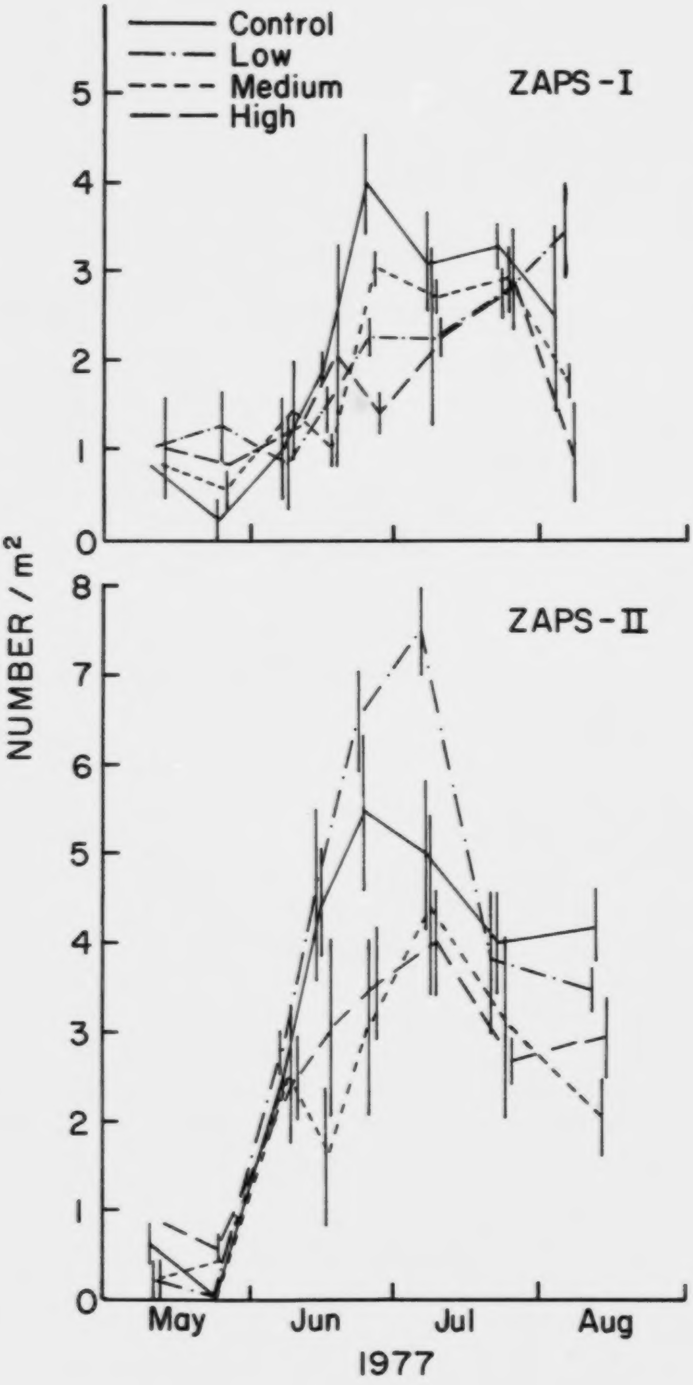


Figure 19.15. Seasonal dynamics of total Acrididae population by treatment ±1 SE.

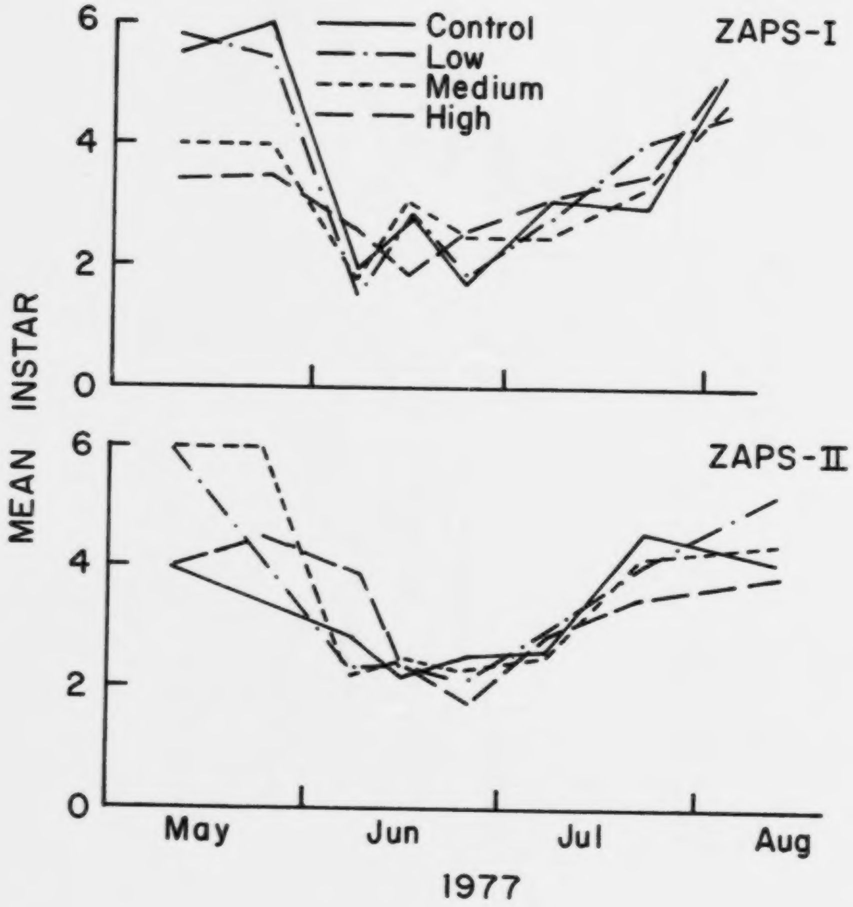


Figure 19.16. Seasonal dynamics of mean instar for Acrididae by treatment.

the leaf to form sulfite $SO_3^=$ and then slowly oxidizes to form sulfate $SO_4^=$ (Ziegler, 1975).

Two species of grasshoppers were used in the feeding trial, *Aulocara elliotti* and *Ageneotettix deorum*. *Aulocara elliotti* was chosen for the trials to complement information obtained from the survival and fecundity study. Toward the middle of the summer it was realized that this species was not going to be common enough on the ZAPS for collection there and the species used was changed to *Ageneotettix deorum*. These two species are closely related and have similar feeding habits (Anderson and Wright, 1952). Both feed heavily on grasses, especially *Agropyron smithii* which is the dominant grass on the ZAPS. *A. smithii* was chosen as the grass used for the feeding trial because it is the preferred grass of the two grasshopper species and much information is known about it and its reaction to SO_2 from auxillary studies on the ZAPS sites.

Late instar nymphs and adult *Aulocara elliotti* were collected from an outbreak area near Springdale, Montana on 20 June 1977. The population there was estimated at 10 to 15 per m^2. The vegetation was similar to the ZAPS but was heavily grazed and *Stipa* spp. grasses were more prevalent. Approximately 400 individuals were collected by sweep net. Some were used in the second attempt to establish the survival and fecundity study, but 50 were kept at the lab for use in the feeding trials. At the laboratory they were separated by sex, placed in cages and fed *Agropyron cristatum*, *A. smithii*, and other grasses collected in the vicinity of the laboratory.

Each feeding trial consisted of placing a number of grasshoppers (usually five or six) in a cage where there was a choice of *A. smithii* from the control and high SO_2 treatment. The food was presented "cafeteria style." A leaf of *A. smithii* was placed in each of ten vials spaced 2.5 cm apart and alternated between leaves from the control and high concentration treatment. Leaves from the control were in positions 1, 3, 5, 7, and 9 and leaves from the high treatment were in positions 2, 4, 6, 8, and 10. Testing was done to determine the length of time grasshoppers should be left in the cage, which was found to be 6 to 8 hours depending on the number of grasshoppers, the temperature, and the time of day. The amount eaten was determined by making a blueprint of each leaf, a method used by Langford (1930), before and after being fed on. The outlines were then cut out and weighed to determine area eaten per grasshopper per hour of each type of grass. A total of eight feeding trials were done with *A. elliotti*, five with just male grasshoppers in the cage and three with just females. Thirteen trials were done with *A. deorum*, eight with grasshoppers collected from the high treatment, five with grasshoppers from the control. One trial was done with *Melanoplus sanquinipes* while looking for a new species to use after finding out populations of *A. elliotti* were too sparse to be collectible on the ZAPS. The data are now undergoing statistical analysis, but preliminary analysis shows a strong rejection of the SO_2-exposed grass in both species of grasshopper (Table 19.12).

TABLE 19.12. WESTERN WHEATGRASS EATEN PER GRASSHOPPER PER HOUR*

Feeding trials involving	Control			High treatment		
	Mean	N	SE	Mean	N	SE
Male *Aulocara elliotti*	2.7	5	0.5	1.5	5	0.7
Female *A. elliotti*	3.5	3	0.7	2.2	3	0.2
All trials using *A. elliotti*	3.0	8	0.4	1.8	8	0.5
Ageneotettix deorum collected from the control	1.6	5	0.2	1.2	5	0.3
A. deorum collected from the high treatment	1.7	8	0.3	1.1	8	0.3
All trials using *A. deorum*	1.7	13	0.2	1.2	13	0.2

*Area (mm^2) from control and high treatments of ZAPS I.

Effects of SO_2 on Survival and Fecundity

Because grasshoppers exhibit considerable trivial movement over an area, a cage study was initiated to insure that certain grasshoppers were exposed to SO_2 over a period of time. The cage design was from Pfadt *et al.* (1970) and measured 20 cm × 20 cm × 51 cm and was made from wood and 32 mesh/inch saran screen. A removable dirt floor was attached to the bottom and served as an oviposition site for adult females and also weighted the cage down to prevent it from blowing over.

In mid-May, 25 first and second instar nymphs were placed in each of four cages on each replicate of the control and high treatment. Within a week all grasshoppers were dead. This could be attributed to many things including overcrowding, higher temperature inside the cage, traumatic shock from movement, and change of diet and other factors.

A second attempt was made during the first week of July when four adults (two males and two females) were placed in each cage. About 25% died within the first week and then the last one died the last week of August. Oviposition was noted and the removable dirt floor was left in the field until 23 September 1977.

The analyses for fecundity and oviposition success are not complete at this time. Preliminary analyses indicate no treatment effects on survival and are too incomplete to indicate anything about the effect of SO_2 on fecundity.

TABLE 19.13. NEMATODE POPULATIONS ON ZAPS SITES, 24 AUGUST 1976*

Nematodes	ZAPS I				ZAPS II			
	Control	Low	Medium	High	Control	Low	Medium	High
Herbaceous	2338	2406	2967	4017	1527	1796	1670	930
Predaceous	1950	1938	1971	1772	2080	1821	1917	1688
Saprophagous	726	1546	1142	934	1455	1528	1055	1645
Total	5014	5890	6080	6723	5063	5145	4642	4263

*Individuals · 17.8 cm^{-2} in surface 15 cm of soil.

NEMATODE POPULATIONS

Nematodes have been shown to be important components of native grassland ecosystems (Smolik, 1974). Their importance stems from their large numbers and subsequent impact on nutrient cycles and energy flow in the belowground part of the system. Smolik (1974) has estimated that nematodes consume as much plant material in northern Great Plains grasslands as do cattle at proper stocking rates.

On 24 August 1976 we estimated the population level of nematodes on the controls and all treatments of both ZAPS sites to determine if there had been a response to the controlled SO_2 exposures (Table 19.13). The population was estimated by taking four randomly located soil cores per treatment. The cores were 4.8 cm in diameter and were taken to a depth of 15 cm.

Preliminary statistical analysis indicates no significant effects by treatment ($P = 0.76$), site × year ($P = 0.105$), feeding type × treatment ($P = 0.71$), or in the feeding type × site × treatment interaction ($P = 0.14$). However, significant differences were found between feeding types ($P < 0.001$) and in the feeding type × site interaction ($P = 0.001$).

In 1977 we intensified the nematode sampling to clarify the trends seen in 1976. One sampling was done on 14 July 1977 wherein 10 soil core samples were taken per treatment (five per replicate) on both ZAPS systems. Each core was 4.8 cm diameter as in 1976; however, the depth was increased to 20 cm. Each core was divided into 0-10 cm and 10-20 cm increments and each section was extracted separately. Extraction was by the Baermann funnel technique (Thorne, 1961). Extraction time was 48 h and efficiency tests were made on a subset of samples. The total nematodes from each core segment were preserved in 50 ml of a 1% Formalin solution. Counting and identification was done by taking three 1.0 ml subsamples and averaging the counts from each then projecting back to the 50. ml total. We categorized the nematodes as stylet bearers (plant feeders), nonstylet bearers (saprophagous), and predators of the genus *Mononchus*. Some of the larger stylet bearers probably were

TABLE 19.14. NEMATODE POPULATIONS ON ZAPS I, 14 JULY 1977*

Nematodes	Depth (cm)	Control	Low	Medium	High
Stylet bearers	0-10	2069	2207	4458	2397
	10-20	1280	1698	1831	2008
	Total	3348(283)	3905(776)	6289(682)	4404(809)
Non-stylet bearers	0-10	2690	2436	2492	1450
	10-20	464	572	633	236
	Total	3154(476)	3008(365)	3125(465)	1686(221)
Total stylet and non-stylet bearers	0-10	4758	4642	6950	3847
	10-20	1745	2270	2465	2243
	Total	6502(663)	6912(1015)	9414(1098)	6090(872)
Stylet/non-stylet ratio	0-10	0.92	0.87	1.84	1.99
	10-20	2.36	3.64	3.78	8.88
	Total	1.19(0.11)	1.31(0.22)	2.14(0.17)	2.93(0.57)
Predators	Total	24.70(7.15)	10.80(1.85)	8.00(3.13)	12.50(3.68)

*Figures are given per core segment or core total; for totals, the standard errors are included in parentheses; the number · m^{-2} = (X)(552.6).

predator, but we could not readily distinguish them, hence the total grouping. Certainly a large majority of the stylet bearers are plant feeders for most of their life cycle.

The total number of predatory nematodes of the genus *Mononchus* per soil core was very low in comparison to the other two categories. Nearly all the specimens of *Mononchus* were found in the 10-20 cm segment of each sample. The data indicate reduced populations of *Mononchus* in the SO_2-treated plots of both ZAPS systems (Tables 19.14 and 19.15); however, the data for ZAPS II are more discrete than ZAPS I. The relatively low counts and high variability may negate a test of significance. The difference in counts of predators from 1976 to 1977 is most likely a result of the difference in expertise of the authors and Dr. James Smolik, a recognized nematologist, who did the extractions and identifications in 1976.

For a majority of the nematodes, the trends, observed in 1976, especially on ZAPS I, were not repeated in 1977. In 1976 there was a substantial increase in plant feeding nematodes with increased SO_2 exposure on ZAPS I (Table 19.13). A similar increase was not noted in 1977. There was a

TABLE 19.15. NEMATODE POPULATIONS ON ZAPS II, 14 JULY 1977[*]

Nematodes	Depth (cm)	Control	Low	Medium	High
Stylet bearers	0-10	3886	3271	3604	3722
	10-20	2086	2879	2005	2446
	Total	5971(863)	6146(712)	5609(635)	6168(442)
Non-stylet bearers	0-10	3975	2526	2283	3091
	10-20	709	695	735	589
	Total	4684(677)	3220(321)	3017(425)	3680(289)
Total stylet and non-stylet bearers	0-10	7860	5796	5886	6812
	10-20	2795	3574	2740	3036
	Total	11,445(1,253)	10,367(921)	8836(917)	9922(653)
Stylet/non-stylet ratio	0-10	1.04	1.51	1.78	1.24
	10-20	3.53	4.35	3.43	4.52
	Total	1.41(0.19)	2.14(0.33)	2.01(0.22)	1.71(0.13)
Predators	Total	15.80(5.67)	20.00(3.49)	4.20(2.26)	1.5(1.4)

[*] Figures are given per core segment or core total; for totals, the standard errors are included in parentheses; the number $\cdot$ m^{-2} = (X)(552.6).

decrease in the saprophagic nematodes on the high treatment of ZAPS I, but at the same time an unusually high number of stylet bearers on the medium treatment of the same ZAPS probably accounts for the trend of increasing stylet bearer/nonstylet bearer ratio. The increase in this ratio on ZAPS I seems valid because it appears in both the 0-10 cm and 10-20 cm levels as well as the total. However, the stylet bearer/nonstylet bearer ratio on ZAPS II did not show as large an increase with increased SO_2 as on ZAPS I, though an increase was noted. The implications for a change in the ratio of the two nematode groups is not clear, but most probably is due to an indirect effect of SO_2 through the vegetation and not through direct exposure to the atmospheric SO_2. These trends, if indeed they are trends, should magnify over time and additional sampling will help clarify them.

TARDIGRADE AND ROTIFER POPULATIONS

The tardigrades and rotifers are microscopic organisms that are considered members of the soilwater fauna, i.e., those organisms living within the thin water films of soil particles (Wallwork, 1970). Very little is known of the biology and ecological relationships of these tiny organisms even though their populations can be very high. Wallwork (1970) indicates the rotifers are vortex feeders, i.e., creating water currents with their cilia and taking in organic material of both plant and animal origin. Tardigrades are thought to feed on a variety of material including organic debris, bacteria, fungi, algae, nematodes, and rotifers. Hallas and Yeates (1972) describe most species of the Tardigrade genus *Hypsibius* as feeding on bacterial films; however, some are considered predatory. The tardigrades collected on the ZAPS sites have been tentatively identified as *Hypsibius* sp. using keys by Schuster and Grigarick (1965). Both the tardigrades and rotifers have the ability to encyst in a state of anabiosis during drought conditions.

The population counts of both tardigrades and rotifers presented here are derived from the same soil samples used for the nematode censusing discussed in the previous section. The Baermann funnel extraction technique has been shown to be efficient for these groups (Hallas and Yeates, 1972). The tardigrades were counted in total for each sample extracted while the rotifers in each sample were estimated by the subsample technique used for nematodes. Generally low counts with high sample variability make it difficult to draw conclusions from the data (a statistical analysis is yet to be run). However, our counts for the 14 July 1977 collection (Table 19.16) show substantial reductions in the populations of both groups in the high treatment plots for both ZAPS. The tardigrades show reduced populations in all but one SO_2-treated plot while the rotifers show population reductions in only the high concentration plots. The mechanism by which these possible reductions are brought about and the significance of the reductions are very difficult to interpret considering the lack of biological information on the two groups. Since both groups are said to be members of the soilwater fauna, the mechanism of SO_2 effect may be by way of the soil microwaterway and not indirectly through the plants. As with the population trends observed for the nematodes and arthropods, the observed trends in the rotifer and tardigrade populations should magnify over time and be clarified with additional censusing.

TABLE 19.16. TARDIGRADE AND ROTIFER POPULATIONS ON ZAPS I AND II ON 14 JULY 1977*

Fauna	ZAPS I				ZAPS II			
	Control	Low	Medium	High	Control	Low	Medium	High
Tardigrade	6.80(2.53)	2.10(0.73)	2.90(0.93)	0.30(0.22)	6.80(3.34)	2.30(1.48)	7.20(4.36)	1.40(0.91)
Rotifers	97(34)	90(21)	95(23)	68(26)	174(35)	177(55)	167(42)	130(23)

*Figures are given per core total. The number $\cdot\ m^{-2} = (X)(552.6)$. Standard errors are in parentheses.

REFERENCES

Anderson, N. L., and J. C. Wright. 1952. Grasshopper Investigations on Montana Rangeland. Bull. No. 486. Mont. Agric. Exp. Stn., Bozeman. 46 pp.

Dodd, J. L., W. K. Lauenroth, R. K. Heitschmidt, and J. W. Leetham. 1977. First-year Effects of Controlled Sulfur Dioxide Fumigation on a Mixed-grass Prairie Ecosystem. In: The Bioenvironmental Impact of a Coal-fired Power Plant, E. M. Preston and R. A. Lewis, eds. 3rd Interim Rep., EPA-600/3-78-021, U.S. Environmental Protection Agency, Corvallis, Oregon, pp. 345-375.

Hallas, T. E., and G. W. Yeates. 1972. Tardigrade of the Soil and Litter of a Danish Beech Forest. Pedobiologia, 12(4):287-304.

Harley, K. L. S., and A. J. Thorsteinson. 1967. The Influence of Plant Chemicals on the Feeding Behavior, Development and Survival of the Two-stripped Grasshopper, *Melanoplus bivittatus* (Say), Acrididae: Orthoptera. Can. J. Zool., 45(3):305-319.

Langford, G. S. 1930. Some Factors Relating to the Feeding Habits of Grasshoppers with Special Reference to *Melanoplus bivittatus*. Tech. Bull. No. 354. Colo. Agric. Exp. Stn., Fort Collins. 53 pp.

Pfadt, R. E., J. E. Lloyd, M. Ali, and G. Sharafi. 1970. Manner of Pickup of ULV Malathion by Grasshoppers from Aerially Sprayed Rangelands. J. Econ. Entomol., 63(4):1210-1214.

Schuster, A. O., and A. A. Grigarick. 1965. Tardigrada from Western North America with Emphasis on the Fauna of California. Univ. California Publ. Zool., 76:1-67.

Smolik, J. D. 1974. Nematode Studies at the Cottonwood Site. US/IBP Grassland Biome Tech. Rep. No. 251. Colorado State University, Fort Collins, Colorado. 80 pp.

Thorne, G. 1961. Principles of Nematology. McGraw-Hill Book Co., Inc., New York. 551 pp.

Thorsteinson, A. J., and J. K. Nayar. 1963. Plant Phospholipids as Feeding Stimulants for Grasshoppers. Can. J. Zool., 41(6):931-935.

Wallwork, J. A. 1970. Ecology of Soil Animals. McGraw-Hill Publishing Co., Ltd., London. 283 pp.

Ziegler, I. 1975. The Effects of SO_2 Pollution on Plant Metabolism. Residue Reviews, 56:79-105.

SECTION 20

SMALL MAMMAL INVESTIGATIONS AT ZAPS: DEMOGRAPHIC STUDIES AND RESPONSES TO GRADIENT LEVELS OF SO_2

J. D. Chilgren

ABSTRACT

A capture-mark-release study of deer mice (*Peromyscus maniculatus*) and prairie voles (*Microtus ochrogaster*) was conducted on two grassland Zonal Air Pollution Systems (ZAPS) at monthly intervals from April to September 1976. Small mammal population structure, biomass, and other functional population attributes were studied in addition to an analysis of the behavioral responses of deer mice to SO_2 at each ZAPS site. Most voles were distributed in a way that precluded a comparable analysis. Animal composition was similar on both ZAPS, although population density and changes were greater or more variable on ZAPS II compared to ZAPS I. In the response analysis, three functional groups of mice were recognized: resident adults and juveniles (group 1); transient adults and juveniles (group 2); and resident and transient adults (group 3). Temporal displacement and numerical replacement of group 1 was directed toward areas of lower SO_2 concentration. Members of group 2, especially juveniles, were trapped more frequently on the control and low SO_2 plots on ZAPS II, but transient juveniles were virtually absent from ZAPS I. Recruitment of group 3 onto the control plots at both ZAPS occurred more rapidly than onto other plots in late summer. Throughout most of the trapping period, the number of occupied traps on all fumigated plots decreased relative to control plots on both ZAPS, and remained relatively higher on control plots from mid-season to the end of all trapping.

INTRODUCTION

In recent years the massive research effort directed toward evaluation of air pollutant effects in vertebrates has emphasized chamber fumigations employing a variety of pollutants that result in acute injury within a short period of time (e.g., hours to days). Many of these studies cannot be applied to ecological models for several reasons. First, exposure rates are frequently too high and therefore unrealistic. These pollutant levels achieved under laboratory conditions are probably seldom attained in nature, with certain exceptions that might, for instance, involve temperature inversions in or near large industrial areas. Secondly, organisms may have inadequate periods of time to adapt to the induced pollutant stress. The homeostatic capability of stressed animals is briefly overcome, thus preventing observations of processes and patterns of individual responses that may result in continued survival or some level of sustained resistance. Thirdly, laboratory animals are normally subjected to optimum living conditions, which maximizes their ability to withstand pollution stress experiments. On the other hand, wild animals are continually exposed to a variety of stressors, each of which presents a challenge to survival. The permutations of the search for food, competition, aggressive interactions, disease, predation, and the vicissitudes of weather and other variables all subvert the animal's ability to counteract additional stresses. Lastly, extrapolations are often made from laboratory test animals to those species whose lives are being tested in nature. Perhaps just as often, no interpretations of laboratory results are given, and the ecological setting that would give them additional meaning is not addressed. These criticisms are not meant to undermine the value and necessity of laboratory experimentation. Controlled laboratory experiments, for example, cannot be valid unless the test populations are of uniform physiological composition. Laboratory tests form the basis from which animal models of pollution-health effects are constructed. Formulation of new hypotheses and testable ideas requires some forecasting from laboratory data. But now that acute exposure data are available, field work should be expected to assume a more distinct role in order to amplify and render more useful the studies now available.

The study reported herein is but one step forward in evaluating small mammal responses to sulfur dioxide (SO_2) over a period of several months in 1976 and 1977. The ZAPS sites provided ideal study areas for these investigations. In addition, this field study was an attempt to bridge the hiatus between acute exposure experiments and quantitative inventories of vertebrates in pollution-impacted areas. Knowledge of subtle changes in population structure may be more helpful in predicting chronic pollution injury or stress than would large population fluctuations related to natural cyclic variation, which may mask those population variables affected by various emissions. Herein lies the difficulty in relating pollutant impact to population dynamics, especially in microtine rodents. Alterations in population levels are governed by a host of variables, several of which cannot be said to exert primary control over these levels. Because non-microtine rodents exhibit fewer amplitude fluctuations than do microtine rodents, their study becomes more significant for the Colstrip project even though the element of population predictability is less well defined than in microtines. Many non-microtine species are widely-distributed and abundant in SE Montana. Both of these factors recommend them for intensive study.

The objectives of this research were to (1) gather population data that would assist in the characterization of small mammal populations in grassland communities, (2) ascertain trends in animal movements within the ZAPS gradient and (3) to determine capture and recapture frequencies at each plot within each ZAPS. Mechanisms by which SO_2 affects grassland rodents may be proposed if deviations in trapping expectancies that may occur at either site are interpreted in the light of current knowledge of SO_2 effects and sensitivity in mammals.

MATERIALS AND METHODS

Trapping Procedure

Both treatment and interval plots were trapped over a six month period in 1976 (Table 20.1). Twenty-five Sherman live traps were placed within each test and control plot in a 5 × 5 pattern with a 15.2 × 17 meter spacing between traps, with 20 traps in each interval plot (plots i_1, i_2, i_3) in a 5 × 4 pattern with distances of 15.2 and 12.2 meters between traps. Plots are graphically represented in Figure 20.1. Traps were prebaited with rolled oats for two days followed by three days of capture-mark-release (CMR). Prebaiting allows free entry into and exit from a baited trap, thus enhancing familiarity to a known food source. Trapped animals were marked by toe clipping and released later at the precise point of capture. Body weight, length, sex, age, reproductive status, pelage color, molt phase, and ectoparasite burden were recorded each time an individual was caught. Age was determined by

TABLE 20.1. 1976 TRAPPING PERIODS

CMR Period*		Day
1. 9-11 April	(prefumigation)	1
2. 13-15 April	(postfumigation)	4
3. 12-14 May	"	33
4. 23-25 June	"	75
5. 28-30 July	"	110
6. 25-27 August	"	138
7. 15-17 September	"	159

*Capture-mark-release.

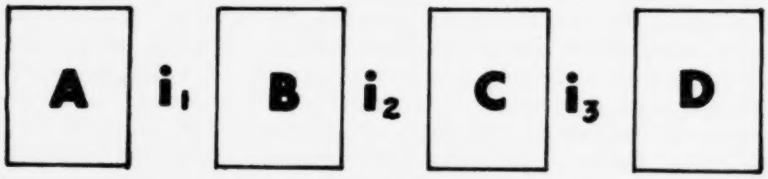


Figure 20.1. A schematic of designated trapping grids at ZAPS I and II.

combined use of weight and length, pelage color, and appearance of the external genitalia. Animals not classed as adults were termed juveniles (see beyond for definitions).

Individually marked traps were opened between 1800 and 2000 hr and checked the following morning on days of CMR. Traps remained open all day during prebaiting periods. Records of both trap number and animal number (as identified by clipped toes) made possible the determination of areas of capture, movement patterns of resident animals, and visitations to individual traps.

After the last day of CMR, live trapping continued for three days (hereafter referred to as trap-out or T.O.). Animals trapped within this period were taken to the laboratory and sacrificed. Blood samples and dissection of major organs were followed by freezing the carcasses for future analysis. These data will be presented elsewhere.

During the 1977 field season, live-trapping of small mammals was conducted on fumigated plots only, and was limited to three trapping periods at three week intervals (27-29 July; 16-18 August; and 6-8 September). One extra day (3 August) of live trapping was conducted to compensate for the low catch during the first trapping period. Blood samples for biochemical analysis were taken from each animal but not exceeding one sample per animal each trapping period. The results from these tests will also be reported elsewhere.

Home Range Estimates

Estimates of home range for mammals captured several times was accomplished by employing the minimum area technique, in which lines joining the outermost points of capture are drawn a map and the area enclosed calculated (Delaney, 1975). Only adults captured at least four times in at least two different months were included in this analysis.

Definitions

Several terms used throughout this section require a brief definition:

1. Adult--a sexually immature animal characterized by adult features including body weight (ca. 14 g) and length minimums, developed sexual or lactating organs, and adult pelage color.

2. Juvenile--a sexually immature animal, not fulfilling the definition of an adult.

3. Resident--an adult or juvenile trapped in successive trapping periods, not necessarily sequential.

4. Transient--an animal trapped only once during a trapping period, and at no time thereafter.

5. Trapping period--two days of prebaiting followed by three of CMR.

6. Activity--the total number of animals trapped, including recaptures, during any trapping period.

RESULTS AND DISCUSSION

Species Composition and Description

The most commonly encountered species were the deer mouse (*Peromyscus maniculatus*) and prairie vole (*Microtus ochrogaster*), both predominantly nocturnal in summer, and the 13-lined ground squirrel (*Spermophilus tridecemlineatus*), a common diurnal rodent. Squirrels were commonly encountered in the spring when traps were opened early or were not checked within three hours after sunrise.

The numbers and proportions of mice and voles trapped at both ZAPS sites over 6720 trap-nights in 1976 were similar (Table 20.2), despite greater canopy coverage and more precipitation at ZAPS I and higher SO_2 levels at ZAPS II (Taylor and Leininger, 1978). Similar to these proportions of animals are the results obtained at Hay Coulee during the 1976 grid trapping study (Chilgren, unpublished data). If the Wyoming pocket mouse (*Perognathus fasciatus*) is ignored for the moment, the catch at Hay Coulee was 75% for *P. maniculatus* and 25% for *M. ochrogaster*. The presence of *P. fasciatus* in reasonable numbers at Hay Coulee (14 of 66) reduced the actual composition of *P. maniculatus* and *M. ochrogaster* to 58% and 20% respectively. Because *M. ochrogaster* prefers denser grass habitats than does *P. maniculatus* (Eadie, 1953) the ungrazed vegetation at ZAPS I and II provided an enriched habitat for *M. ochrogaster*. The latter is found throughout all areas of Powder River and Rosebud Counties where adequate cover and grass exist. Heavily grazed areas appeared to be consistently low in vole numbers.

TABLE 20.2. SPECIES COMPOSITION AT TRAPPING SITES*

Site	*P. maniculatus*	*M. ochrogaster*	Other	Total
ZAPS 1	86 (76.8%)	26 (23.2%)	0	112
ZAPS 2	101 (71.1%)	38 (26.8%)	3†	142

*Numbers exclude squirrels; numbers in parentheses are percent of total.

†Includes one *Reithrodontomys megalotis*, one *Perognathus fasciatus*, and one *Thomomys talpoides*.

Sex and Age Composition

The distribution of sex and age classes of *P. maniculatus* at ZAPS I and II is shown in Figure 20.2. The sex classes throughout the trapping season are shown for *M. ochrogaster* (Figure 20.3). Only two juvenile deer mice were trapped at ZAPS I for the period April-August 1976, while 17 were caught at ZAPS II during this time. Further division of sex classes of voles into age

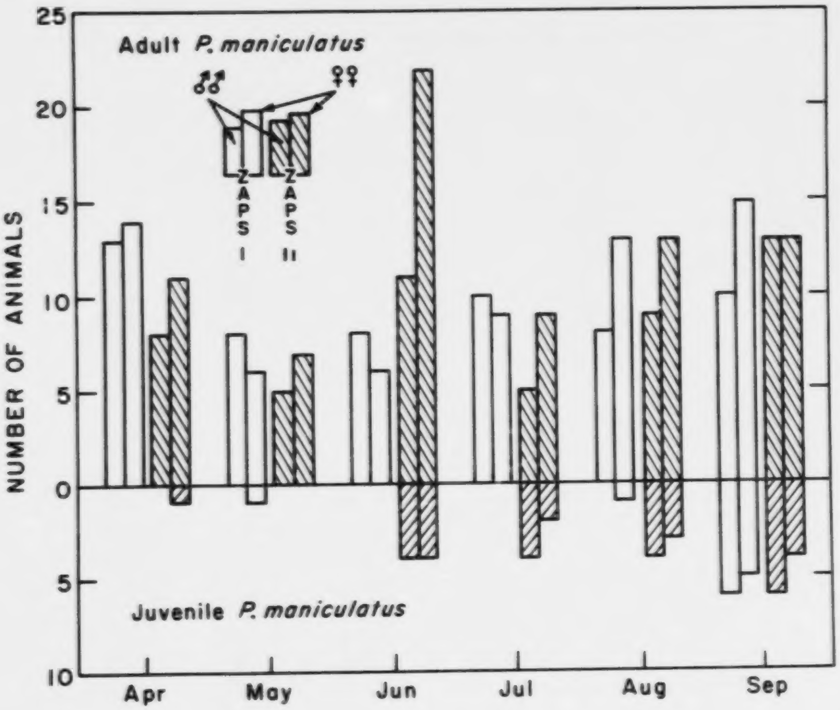


Figure 20.2. Sex and age composition of *P. maniculatus* at ZAPS I and II on all plots from April to September 1976.

classes seems unnecessary here because the response of voles to the ZAPS SO_2 gradient appeared to be undeterminable. Nearly all voles ostensibly inhabited plot B and its adjacent interval plots (see Figure 20.4). No voles were caught in Plot A and very few in plot D. The reasons for this clumped distribution of voles on both ZAPS sites are unknown. Although voles form colonies of seasonally changing densities, the remarkable and fortuitous similarity in colony location appears for the present to be only coincidental. For example, a vole colony was found at plot D on ZAPS II in 1977, and hence I assume that there was nothing peculiar to plot B at both sites that attracted them. The existence of vole colonies on plot B did facilitate intersite comparison and was a prominent factor in establishing experimentally one site as a spatial replication of the other. The nonuniform distribution of voles, however, did not permit any correlation between SO_2 level and vole behavior. The extent to which voles may have interferred with deer mouse emigration is not known. However, few mice were ever trapped in areas containing large vole colonies. This apparent exclusion occurred mainly on the north end of plot B of ZAPS II in 1976, and was tested statistically using a 2 × 2 frequency analysis for both ZAPS sites. In each analysis, a negative association between *Peromyscus* and *Microtus* was detected, being stronger on ZAPS I ($P < 0.001$) than on ZAPS

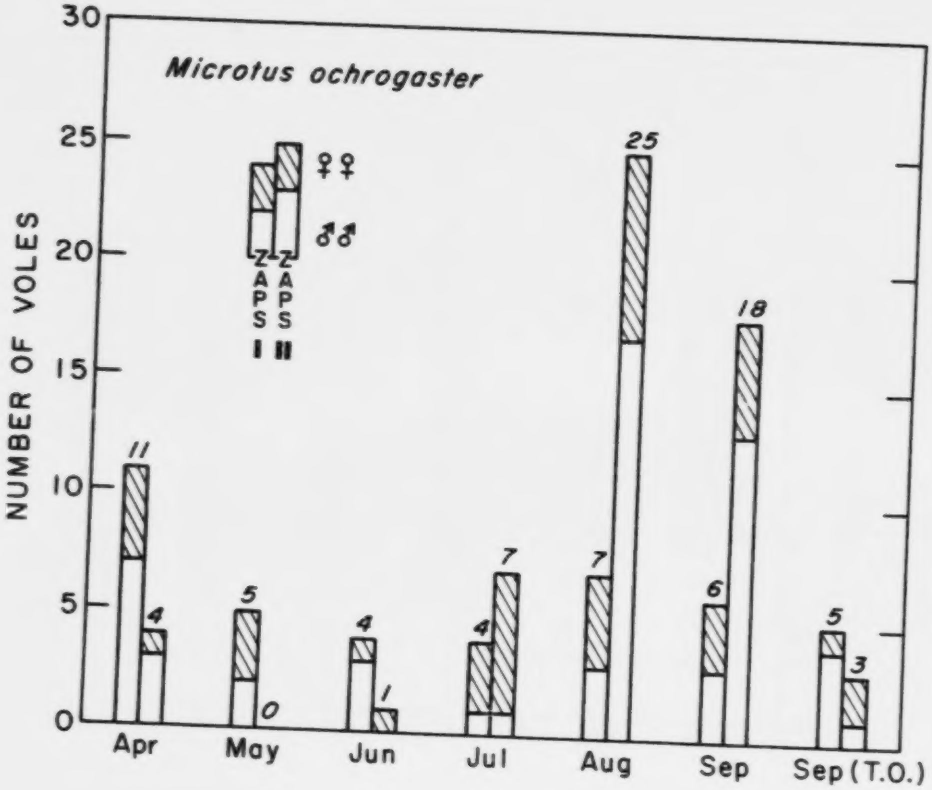


Figure 20.3. Sex composition of *M. ochrogaster* on ZAPS I and II on all plots from April to September 1976. T.O. indicates trap-out: animals removed after the last date of CMR.

II (P < 0.025). Hence, these species were not distributed independently of each other.

Population Characteristics

It was not critical among the objectives of this experiment to characterize the population levels at ZAPS. First, trap distances were not conducive to population analysis and no removal trapping was conducted. Nevertheless, it is possible to characterize the population in general terms and to make some specific statements as to the degree of similarity in population structure at both ZAPS. Sex and/or age composition of *Peromyscus* and *Microtus* has already been shown (Figures 20.2 and 20.3). Populations levels of *P. maniculatus* varied throughout the season, reaching zenith levels at both ZAPS in the period from mid-summer to fall. This late peak was correlated with the appearance of juveniles in the existing population. Because the SO_2 appeared to be a strong factor in organizing the distribution of *P. maniculatus*, it is inappropriate to attempt an accurate population estimate. Generally speaking, however, population levels at ZAPS I were more uniform, varying at the most

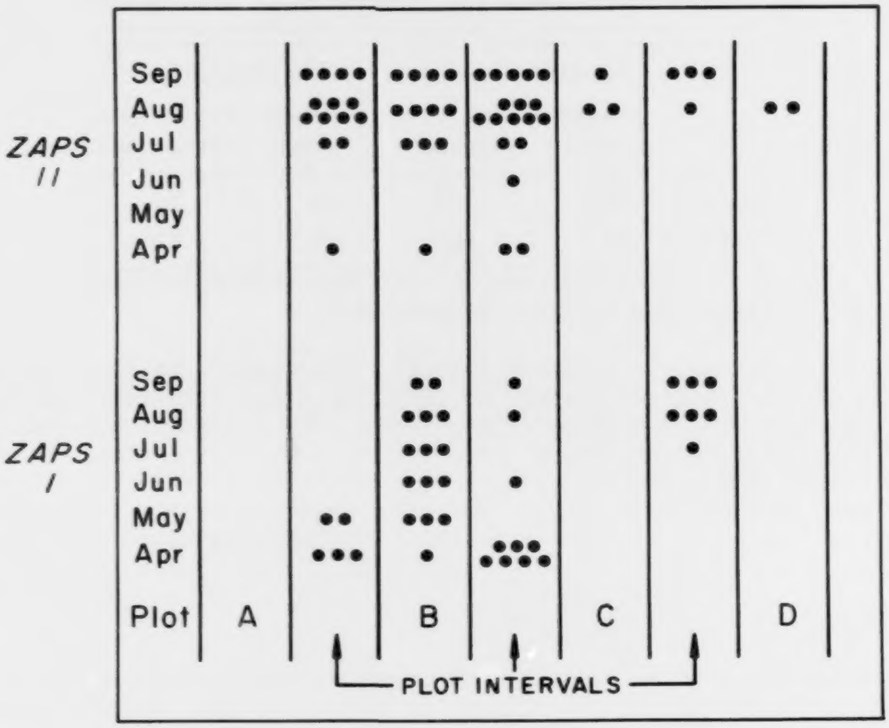


Figure 20.4. Distribution of *M. ochrogaster* at ZAPS I and II from April to September. A dot indicates one individual.

during the summer by a factor of about 1.8, whereas at ZAPS II, this factor was about 2.7. Refined and elaborate techniques of population density estimates were not employed because density analysis was not central to the study objectives. Furthermore, mark-recapture methods suffer the weakness of equal trappability assumptions, the fallacy of which cannot be detected or compensated for statistically. Removal techniques are superior and, as mentioned above, were not conducted under the experimental design of the ZAPS small mammal study.

Table 20.3 summarizes the recapture data at both ZAPS sites. The mean recaptures per individual are 3.2 and 2.8 for *Peromyscus* and 2.6 and 1.5 for *Microtus* at ZAPS I and ZAPS II, respectively. The values for *Peromyscus* are about 30% higher than those obtained at trapping grids near Colstrip (Morton and Chilgren, in press), although they are not without bias. On both ZAPS, there were two mice caught 21 times and two voles caught seven and 16 times. Obviously animals caught early in the season had a chance of being trapped much more often than those trapped later, say in July.

Transient animals often form a large fraction of trapped populations (Terman, 1968). Transient *P. maniculatus* accounted for 38% and 52.6% at ZAPS

TABLE 20.3. RECAPTURE SUMMARIES AT ZAPS SITES*

	P. maniculatus		*M. ochrogaster*	
Site	Number caught	Total times recaptured	Number caught	Total times recaptured
ZAPS I	76	242	21	54
ZAPS II	93	260	35	53

*Excludes trap-out individuals.

I and II, respectively. Animals captured in September could not be considered as transients or residents, since no trapping occurred in later months to verify the status of a September individual. The transient population appeared to be affected by the SO_2 levels (see page 777). A transient individual by definition could have been trapped one to three times during a trapping period but could not be caught at any other trapping period. This definition has limitations since a trap-shy resident trapped only once would be erroneously called a transient.

Survival

Because the population could not be determined accurately, it is not possible to determine mortality rates of mice. However, one can compute a probability curve defining appearance and disappearance rates without knowing specific population numbers. The data used as a basis for this curve are shown in Table 20.4. The "Total Animals" column summarizes the number of

TABLE 20.4. PROBABILITY OF APPEARANCE DATA FOR ZAPS I AND II*

Site	Month	Total Animals	Chances	Probability
ZAPS I	1	31	31	1.00
	2	16	24	0.67
	3	9	18	0.50
	4	5	14	0.36
	5	4	10	0.40
ZAPS II	1	34	34	1.00
	2	21	27	0.78
	3	13	25	0.52
	4	4	15	0.27
	5	3	13	0.23

*See text for explanation.

animals known to be alive each month since their initial release month (April to August) and excludes animals that died in traps or that suffered some trauma during the capture and marking process. Because mice caught later in the season do not have an equal chance of being captured as do mice caught early in the season, the chances of capture for all individuals is summarized under "Chances," and the ratio of animals captured to the chances of capture (Probability of Appearance) defines the proportion of recaptured mice known to be alive for a subsequent month. The month of initial release is month 0 and subsequent months are numbered 1 to 5. For an animal trapped in August for the first time, there would be only one subsequent month (September). The probability values are then plotted on semilog paper and a logarithmic function is calculated to define the curve (Davis, 1964). The last month values can distort the curve since the number of animals appearing may not decline as fast as the chances of capture. Hence, the last month for ZAPS I has been omitted so that a more reliable probability curve could be drawn. The expressions for these functions on each site are as follows:

$$(1)\quad \text{ZAPS I:}\quad \log y = 0.136 - 0.147x \; (r^2 = 0.996)$$
$$(2)\quad \text{ZAPS II:}\quad \log y = 0.218 - 0.179x \; (r^2 = 0.968)$$

where y is the Probability of Appearance (the number that appear divided by the initial population) and x is the month of capture after marking the initial population. The annual probability of appearance can be calculated by substituting 12 for x and computing y. For ZAPS I y is 0.023 and for ZAPS II y equals 0.012. The probability of disappearance is 1 - y. Hence, the probability of disappearance is 0.977 and 0.988 for ZAPS I and II respectively. These values indicate that the chance of recapture after one year are comparably small at both sites, which is not unexpected for Cricetid rodents (French *et al.*, 1975). These numbers might be liberally interpreted to indicate that the survival rate is rather low, and that the lifespan of a mouse is not likely to exceed one year at this particular locality. The extent to which SO_2 may have affected these calculations cannot be determined. It should be mentioned here that five *P. maniculatus* trapped in April were at ZAPS I and four at ZAPS II in April were also trapped in September. Hence, the known lifespan of some mice in this region is at least 7-8 months (six months seen in trapping and one to two months for maturity and development).

Home Range

The home range is the area over which a small mammal will travel in the course of its normal or routine activities. Home ranges were calculated only for adults and those mice that were captured four or more times in at least two different trapping periods. Animals captured in successive months and that appeared to have shifted their apparent home range (Brown, 1966; Stickel, 1968) were also not included. Because of the fumigation, animals may not have lived on the grids, but merely may have been attracted to the oat bait in the traps. However, since home range calculations are estimates at best, it is reasonable to compare these data with existing data to determine the overall reliability of the estimates. Table 20.5 shows home range estimates for both *P. maniculatus* and *M. ochrogaster*.

TABLE 20.5. HOME RANGE ESTIMATES FOR RODENTS IN CUSTER NATIONAL FOREST*

	P. maniculatus						*M. ochrogaster*		
Site	Male	N	C†	Female	N	C	Both sexes	N	C
1	0.22 ± 0.09	14	9	0.12 ± 0.07	15	6	0.11 ± 0.07	7	7
	(0.54 ± 0.22)			(0.29 ± 0.18)			(0.27 ± 0.17)		
2	0.14 ± 0.05	12	8	0.12 ± 0.05	18	8	0.05 ± 0.12	10	5
	(0.33 ± 0.13)			(0.30 ± 0.14)			(0.14 ± 0.04)		

*Mean area (hectares) ± S.D.; acres in parentheses; N = number of animals.

†Average number of captures per individual.

The existing literature on *Peromyscus* reveals that the home range size of most species of adult males is larger than that of females. Stickel (1968) has summarized home range sizes for several subspecies of *P. maniculatus* and indicates than in six of these, the male home ranges are larger than in females, and in two subspecies the sizes are equivalent. Because I believe that *osgoodi* is the subspecies at ZAPS, there are no comparable data, but even so the data in Table 20.5 are somewhat confounding. Male *maniculatus* home ranges were significantly larger ($P < 0.005$) than that of females at ZAPS I as well as that of males at ZAPS II ($P < 0.01$). Female home range estimates were comparable at both sites. Nevertheless, the estimates for *Peromyscus* in Table 20.5 are well within prior estimates for other species and subspecies of *Peromyscus* (see Table 1 in Stickel, 1968). These estimates appear to be less than those calculated by Metzgar (1973), however, for *P. maniculatus* near Missoula, Montana. He used a much larger (3.2 ha) grid and calculated home range size for both feeding and exploratory activity. Measured from the animal's center of activity, the mean distances for feeding and exploratory activity were 30.6 and 31 m, respectively. If transformed into circular areas, his home range data exceed those of male mice in Table 20.5 by 30-50%. Since grid areas and methods of measurement were different in both studies, and the data in Table 20.5 exhibit some deficiencies (*e.g.*, low averages of captures per animal), these differences do not seem incompatible. Furthermore, Metzgar may have been experimenting with a different subspecies whose habitat was quite dissimilar from that of the ZAPS sites.

Although the home range estimates for *M. ochrogaster* appear to be different the 95% confidence limits calculated for means at each site overlap (ZAPS I: 0.052-0.164; ZAPS II: 0.044-0.066) and are therefore not significantly different. Using a modified minimum area technique that reduces the magnitude of the original technique, Harvey and Barbour (1965) estimated the home range of six *M. ochrogaster* to be 0.09 acre, or about 43% that of the unmodified technique. Assuming the true home range of *M. ochrogaster* to be overestimated by the technique I employed and by applying a correction factor derived from Harvey and Barbour, the average home range for 17 voles at both ZAPS is computed to be 0.06 acre at ZAPS II and 0.11 acre at ZAPS I. These values agree with those of Harvey and Barbour, and are compatible with an average value of 0.075 acre for *M. pennsylvanicus* obtained with an isotope technique (Ambrose, 1969).

Home Range and Biomass: Relationships to Population Density

Home range can be expected to change in response to physical factors (season and weather), population factors (population density), ecological factors (habitat and food supply) and an individual's biology (sex, age, and behavioral patterns). Because home range estimates were made at the same time of year and under conditions of similar habitat and weather, the only conspicuous factor named above that differed extensively at ZAPS I and II was population density. Juveniles were virtually absent from ZAPS I until August unlike ZAPS II, on which several juveniles were trapped from June until the end of the trapping period in September. Expressed in terms of biomass (grams per hectare) or the total mouse and vole weight per unit land area, the population density at ZAPS II peaked in June owing ostensibly to juveniles entering the population from within (recruitment) as well as adults and juveniles entering from peripheral areas (emmigration) (Figure 20.5). No comparable change was observed at ZAPS I. The magnitude of the differences in biomass is expressed in Table 20.6 as a ratio for each month of trapping. Thus, both the rate of population increase as well as the population at peak level may be correlated with the lower estimate of male home range at ZAPS II, since population density and home range size are often inversely related (Stickel, 1960). Although I have no evidence that other variables may not have been operative, the data suggest that population density may be one feature regulating home range since a similar pattern was observed in *M. ochrogaster* (Figure 20.5). These data indicate that vole biomass increased dramatically at ZAPS II but was much less variable at ZAPS I. The mean home range estimate for *M. ochrogaster* was more than halved, although the high standard deviation at ZAPS II reduced the level at which confidence intervals are nonoverlapping to less than 90%. Nevertheless, there is a clear trend expressing the relationship between population density and home range estimates. This discussion takes no note of the possibility that unknown differences in species trappability may have adversely affected the above analysis. One has to make this assumption in order to evaluate the available data. I also cannot explain why female home ranges did not vary if population density is affecting home range of both sexes.

Experimental Effects

Assumptions and Hypotheses

The following assumptions were made in the interpretation of these results. First, terrain and habitat features are similar on all plots at both ZAPS so that habitat preference is assumed to not influence the results. Second, the chances that an animal may traverse a plot or be trapped within any one plot are assumed to be equal for all plots.

The null hypothesis is that immigration or recruitment onto any portion of the ZAPS proceeds unimpeded as if no SO_2 were present. On the contrary, if a mouse or vole is sensitive to SO_2 at these concentrations, its response to test plots should be different in space and time from that of control plots.

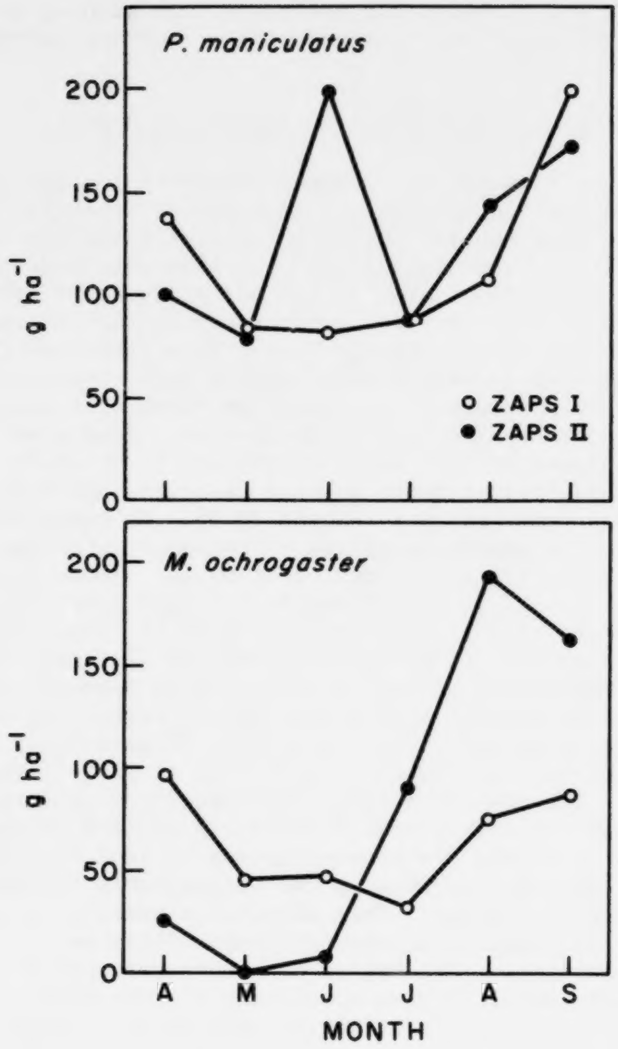


Figure 20.5. Biomass changes from April to September 1976 in two common rodent species in SE Montana.

TABLE 20.6. RELATIVE BIOMASS DIFFERENCES OF MICE AND VOLES*

Species	Apr	May	Jun	Jul	Aug	Sep
P. maniculatus	0.73	0.94	2.44	1.00	1.32	0.87
M. ochrogaster	0.27	0.00	0.20	2.82	2.54	1.84
Both species	0.54	0.61	1.60	1.49	1.83	1.17

*The quotient of ZAPS I biomass/ZAPS 2 biomass.

Categories of Experimental Animal Groups

Three functional groups of mice were recognized: resident adults and juveniles (residents), transient adults and juveniles (transients), and resident and transient adults (adults), a group created by necessity because of the low incidence of juveniles on ZAPS I. As juveniles are often dispersing to find new territories, a category of resident and transient juveniles as a separate entity did not exist and I found no evidence that juveniles as a group took up residence at either site. These groups of mice form the basis of the subsequent discussion. Voles have been excluded from discussion, except where their presence affected mouse populations, for reasons given previously.

Capture Patterns of Resident Mice

Because the burrow sites of resident mice were not determined, a mouse that lived within a fumigated plot could not be distinguished from one that lived outside the plot but returned to the trap for food. As no trapping was conducted beyond the borders of test or interval plots, no movement of resident mice other than that parallel to and within the gradient could be ascertained. The criteria for movement within this zone were (1) capture at least twice in the area of initial capture followed by successive captures in another interval or plot at least 45 m away; and (2) capture in the new area at least once in a future trapping period.

At ZAPS I, 16% (eight mice) of all mice trapped (26% of all residents) fulfilled these criteria, and at ZAPS II these figures were 5.3% (four mice) of all mice trapped (11.1% of all residents). Of these 12 mice, seven moved down and five up the gradient. Six of the seven moving down were initially trapped on plot D and one of the adjacent intervals. Of the five moving up the gradient, three originated at plot A, one at plot B, and one at plot C. None of these moved into plot D. At least two explanations are possible. First, if the burrows of permanent residents were not within the ZAPS, these mice may not be affected by exposure to SO_2 while entrapped. The lure of plentiful food then outweighed any discomfort a mouse experienced during its stay within test plots. It is equally possible that those residents showing movement may have had their burrows on the ZAPS and moved accordingly in response to SO_2. Second, if most residents had burrows within the ZAPS, they may not have been sufficiently repulsed by SO_2 to emmigrate on a large scale. In addition, the number of residents having burrows within the ZAPS that emigrated shortly after exposure to SO_2 in April cannot be determined. If captured only once, these mice are by definition transients. Any number of mice that were so misclassified would tend to weaken the argument that only a small percentage of residents moved out of the SO_2 field.

Temporal Capture Patterns of Resident and Transient Adults

The temporal changes in adult mice trapped on each ZAPS site (recaptures excluded to minimize bias associated with trap-shyness or trap-addiction) are depicted in Figure 20.6. Juvenile mice have been omitted because of the profound discrepancy between numbers of juveniles trapped on ZAPS I and on ZAPS II. Their omission facilitates a more equitable comparison of mice of all age

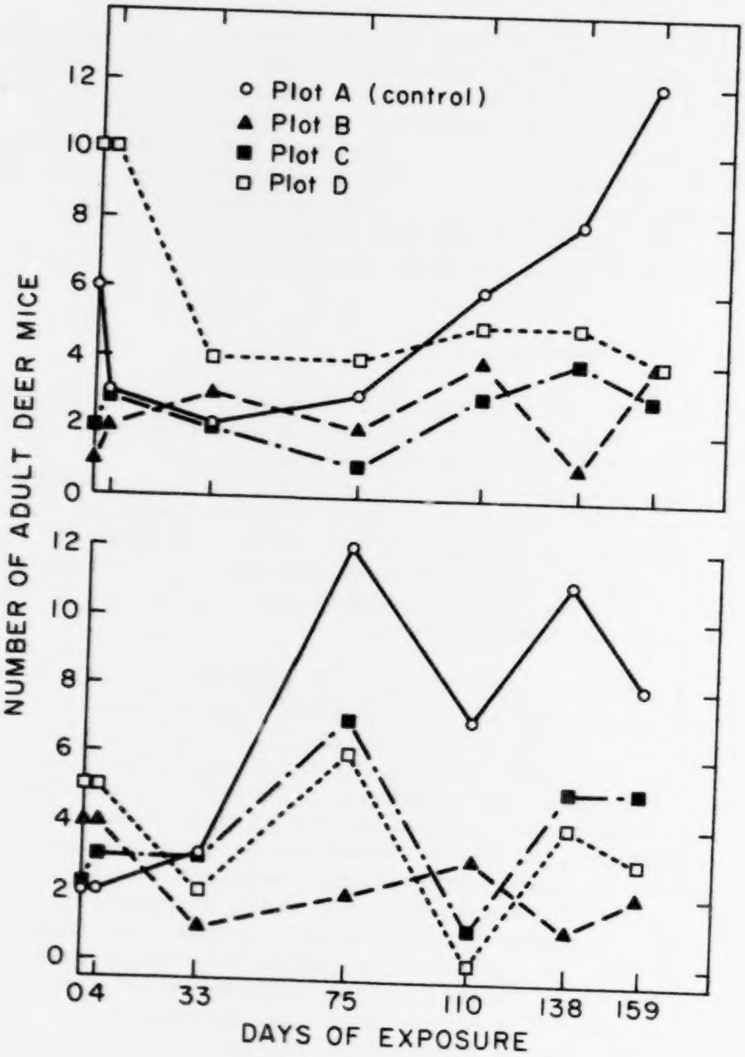


Figure 20.6. Temporal changes in numbers of adult *P. maniculatus* on test plots at ZAPS I (top) and II (bottom).

groups. These graphs show that while mice on plots C and D are relatively stable and low, control plots show enhanced recruitment throughout the season, especially at ZAPS I. The low numbers of mice observed on plot B can be attributed to the presence of voles. It can also be shown that totals of adult mice summed May to September differ significantly on plots A, C, and D, with plot B excluded for the reason just given (Table 20.7). April is not included because of the three days between initial and postfumigation CMR periods were thought to be insufficient for SO_2 to achieve a noticeable effect

TABLE 20.7. X^2 ANALYSIS OF SEASON TOTALS OF ADULT MICE (MAY TO SEPTEMBER)

Site	Plot A Observed	Plot A Expected	Plot C Observed	Plot C Expected	Plot D Observed	Plot D Expected	X^2
ZAPS I	31	14.4	13	18.6	22	14.4	24.6*
ZAPS II	41	18.9	21	21.6	15	16.9	34.8*

*$P < 0.001$.

on animal populations, although data collected in April at ZAPS II indicate that effects may have been felt within this period of time (see page 782).

Capture Patterns of Transient Mice

The ZAPS SO_2 gradient should exert no effect upon the transient population if the assumptions mentioned above are valid. One may expect, therefore, approximately equal numbers of transients on all plots, although there may be unbalanced numbers in both sex and age classes (Terman, 1968). Because fewer transients were trapped on ZAPS I, the following analysis has been simplified by treating the control and low plots and interval i_1 as one section (Ai_1B) and plots C and D and interval i_3 as another (Ci_3D). Animals in the middle interval were assigned to one unit or another, depending on proximity of capture to either unit.

Several hypotheses were tested in this analysis.

1. The numbers of adult and juvenile transients trapped in section Ai_1B should equal approximately those trapped in section Ci_3D.
2. The numbers of adult transients should be comparable on plots A and D.
3. The numbers of juvenile transients should be comparable on plots A and D.
4. Juvenile transients should be evenly distributed across each ZAPS.
5. Adult transients should be evenly distributed across each ZAPS.

The results of this analysis are summarized in Table 20.8.

ZAPS I and II are dissimilar with respect to hypothesis 1, and hypotheses 3 and 4 are not supported at ZAPS II. An effect of SO_2 on the behavior of transient mice, especially juveniles (compare hypotheses 4 and 5) is indicated. However, many juveniles were trapped on all plots in September, but because no trapping was conducted in October, these mice cannot be classified as transients or residents. Hence, the hypothesized SO_2 sensitivity difference between adults and juveniles cannot be fully supported. Another unexplained observation is that with one exception in May all transients trapped in section Ci_3D at ZAPS II were caught in June, whereas on section Ai_1B they were trapped at all times, except in May when none were caught. No comparable data exist for ZAPS I except in September, thus precluding any deductions

TABLE 20.8. TRANSIENT MOVE[illegible]ND DISTRIBUTION

Hypothesis number	Site	Data	[illegible] P	Hypothesis supported
1	ZAPS I	9, 10	0.[illegible], 0.26	Yes
	ZAPS II	29, 12	7[illegible]7, 0.99	No
2	ZAPS I	7, 5	0.42, 0.48	Yes
	ZAPS II	13, 7	1.85, 0.83	Yes
3	ZAPS I	---	---	insufficient data
	ZAPS II	12, 0	10.1, 0.95	No
4	ZAPS I	---	---	insufficient data
	ZAPS II	19, 8	3.70, 0.95	No
5	ZAPS I	13, 12	0.08, 0.22	Yes
	ZAPS II	15, 13	0.18, 0.33	Yes

based upon independent observations in time. In this foregoing analysis, the presence of voles has been ignored, but their presence on plots B, i_1, and i_2 serves to accent the difference observed with regard to hypotheses 1, 3, 4, and to some extent 2 because of the presumed increase in mice trapped at plot B in the absence of voles.

Capture Patterns at Individual Plots

For each plot, animal activity (see definitions, page 767) was summed throughout all trapping periods for both peripheral and interior portions of each fumigated plot as well as for each interval. Peripheral portions were the 16 traps forming the boundary of each plot, and the remaining nine traps forming the interior portions. Two hypotheses were tested. First, peripheral activity of all plots should be comparable, and second, interior activity of all plots should be comparable as well. Both of these hypotheses assume that a large number of mice live outside each ZAPS and are attracted to the bait. If each ZAPS is regarded for the moment as a large rectangular grid, the boundary of this grid is the boundary of traps around each ZAPS. Table 20.9 shows that an edge effect was present and that these hypotheses are most likely valid. This table shows that the total activity in the peripheral boundary of the entire ZAPS (which contained 44% of the total traps) was significantly greater than that of the interior portion (containing 56% of all traps). It also suggests that each plot might show a similar tendency, and if both hypotheses are valid, boundary traps can be expected to "filter" immigrants or trap visitors and should fill at a more rapid rate than interior traps. Then, if SO_2 does not affect foraging behavior, the interior traps at each plot should also fill at equivalent rates. The data pertaining to activity at the peripheral and interior regions of each plot are shown in Table 20.10.

TABLE 20.9. ANALYSIS OF THE EDGE EFFECT FOR DEER MICE*

Site	Total activity		% of total activity	
	Peripheral†	Interior†	Peripheral	Interior
ZAPS I	179	110	61.9	38.1
ZAPS II	202	124	62.0	38.0

*Summed throughout all trapping periods; excludes mice known to have died during a CMR session.

†See text.

TABLE 20.10. REGIONAL ACTIVITY SUMMARIES AT EACH ZAPS PLOT

Site	Plot and region							
	A		B		C		D	
	P	I	P	I	P	I	P	I
ZAPS I	40	30	35	7	33	14	42	24
ZAPS II	55	28	48	11	48	14	48	7

There was no statistically significant difference in peripheral activity among plots ($X^2 = 5.95$, $P > 0.10$ for ZAPS I, and $X^2 = 0.74$, $P = > 0.60$ for ZAPS II). Plot B was eliminated in these goodness-of-fit tests for interior activity because of the apparent inhibitory effect of voles on deer mouse distribution. While peripheral activity was shown to be similar among all plots, interior activity was reduced on plots C and D of ZAPS II ($X^2 = 15.22$, $P < 0.01$) but not on ZAPS I ($X^2 = 5.96$, $P > 0.10$) owing to results at plot D. When the effect of time is considered, however, it can be shown that 42% of the interior activity on ZAPS I occurred during the first three days of fumigation in April. If the April figures are not included, the values for I in Table 20.10 then become 27, 6, 8, and 16 for the respective plots of A, B, C, and D ($X^2 = 27.4$; $P < 0.01$).

Temporal Changes in Relative Activity

Figure 20.7 illustrates the seasonal changes in numbers of transient and resident adult and juvenile activity within each experimental plot relative to the control plots. At both ZAPS sites, there was a downward trend on all three experimental plots after the initial surge of activity, which might be explained by the initial exposure of each ZAPS to SO_2. The relatively low levels of activity in plot B can probably be attributed to the presence of voles. Because this pattern is duplicated spatially, it is unlikely that variables other than SO_2 could exist simultaneously at both sites and elicit the same pattern. These data then provide some of the strongest evidence for the effects of chronic low levels of SO_2 upon animal behavior.

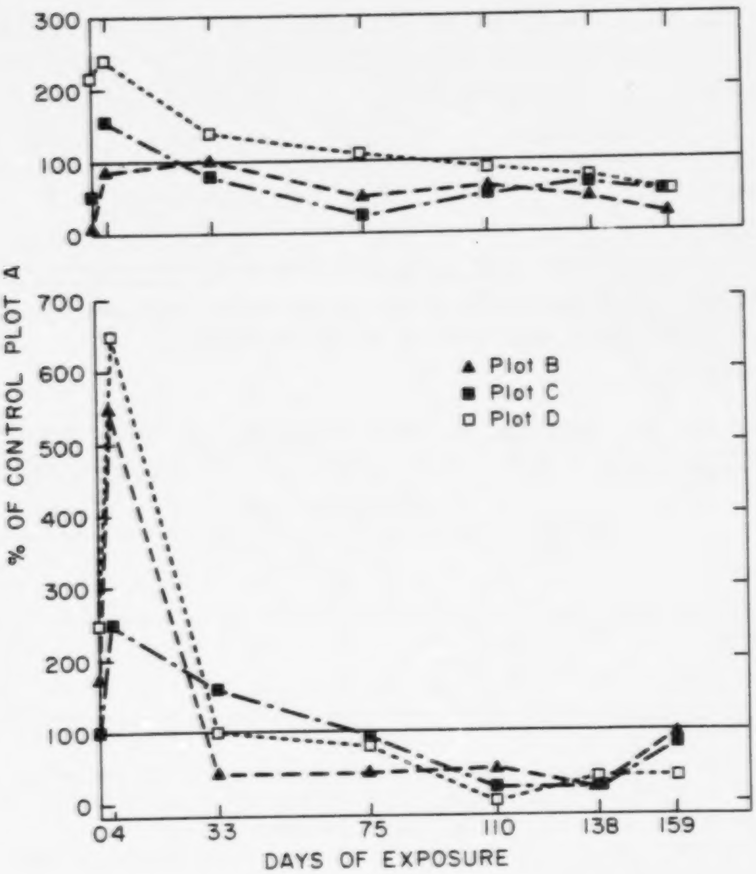


Figure 20.7. Temporal changes in relative activy of *P. maniculatus* on test plots at ZAPS I (top) and II (bottom).

Correlation Between Activity and SO_2 Levels

Hourly means of SO_2 (pphm) were averaged over 12 hr periods (1800 to 0600) on nights of CMR. Averages for all three nights of each trapping period were computed and examined against the activity of deer mice. No correlations were apparent. These results are expected for several reasons. First, SO_2 levels often varied substantially throughout each 12 hr period. Averaging SO_2 levels obscures real differences between hours or blocks of hours that might have correlated with activity if the times of capture were known. Second, any correlation would imply an immediate behavioral response to SO_2. Although it is not known how *P. maniculatus* initially responds to SO_2 inhalation at the levels experienced in the field, the results of this experiment show that some mice may tolerate SO_2 levels for long periods. For example, some mice at both ZAPS were trapped each month or nearly every month. Hence, it would appear that in addition to any direct effect of SO_2 on an animal, there may be indirect effects as well operating through various pathways, *e.g.*, food and water quality or abundance, or habitat characteristics. Lastly, the *ground* levels of SO_2 concentration actually inhaled by nocturnal ground-dwelling animals were not measured and probably did not resemble the seasonal mean concentrations on each plot. Sulfation plate data (see Section 10) indicate that SO_2 levels peak at canopy height, decreasing above and below it. However, inversion conditions in the presence of little wind seems to have been a common pattern according to SO_2 levels monitored each night. This situation allowed SO_2 levels to increase by an order of magnitude or more beyond the seasonal averages. Clearly, this suggests that nocturnal rodents may have been exposed to irritating levels (ppm range) of SO_2, although this effect may have been attenuated at the ground level. Thus, the lack of correlation between activity and SO_2 level is explained.

Field Operations in 1977

At ZAPS I, six adult male and six female (four adult, two juvenile) *P. maniculatus* were marked along with three *M. ochrogaster* (one adult male, two adult females). At ZAPS II, eight adult female and five male (three adult, two juvenile) *P. maniculatus* were marked. The catch for *M. ochrogaster* was eight males (four adults, four juveniles) and eight females (five adults, three juveniles). The respective distribution and activity of both species at each site are summarized in Tables 20.11 and 20.12. These data do not differ in substance from that described for the 1976 experiment. It is evident that activity is more prominent on plots A and B. Assuming homogeneity of sam-

TABLE 20.11. TRAPPING RESULTS FOR *P. maniculatus* IN 1977

		Plot			
Site	Variable	A	B	C	D
ZAPS I	Total animals	6	2	0	4
	Activity	17	10	0	8
ZAPS II	Total animals	7	1	2	3
	Activity	19	5	6	6

TABLE 20.12. TRAPPING RESULTS FOR *M. ochrogaster* IN 1977

		Plot			
Site	Variable	A	B	C	D
ZAPS I	Total animals	2	1	0	0
	Activity	4	1	0	0
ZAPS II	Total animals	3	4	3	6
	Activity	3	6	9	10

pling, activity at ZAPS I for *P. maniculatus* is significantly greater on plots A and B compared to plots C and D ($X^2 = 0.26$; $P < 0.005$), but not on ZAPS II ($X^2 = 3.36$; $0.10 > P > 0.05$) owing ostensibly to insufficient numbers of trapped animals.

M. ochrogaster, like *P. maniculatus*, appeared in insignificant numbers on ZAPS I to withstand any critical evaluation. Voles were found on all plots at ZAPS II, and there appeared to be three distinct areas where they could be found. These were the upper zone of plot B, lower end of plot C, and the eastern half of plot A. On the basis of the previous years work, vole colonies appear to have been established in new areas. Based on trapping efforts their virtual absence from ZAPS I in 1977 is not readily explained, however.

Species Differences and Problems in Field Studies

Several lines of evidence presented in this report indicate that chronic low levels of SO_2 influence the abundance and activity of free ranging populations of deer mice. This study produced insufficient evidence that the prairie vole may be similarly affected. It is now known that *M. ochrogaster* is especially resistant to pesticide toxicity compared to other vole species (J. Gillett, pers. comm.). Furthermore, another species, *M. pennsylvanicus*, has been shown to be resistant to the long term toxicological effects of the pesticide endrin at levels where *P. maniculatus* populations never recover (Morris 1970). The deer mouse may therefore be a better candidate for assessing low-level air pollution than the vole, a judgment felt appropriate in view of the wide distribution of this species throughout the United States.

The modes of application of these small mammal studies to air pollution impact assessment are not readily obvious. Although changes in behavior are indicated as a bona fide response to SO_2, the effect was not correlated with the median SO_2 levels and was complicated by the presence of vole colonies on plot B. Furthermore, there are multiple factors that influence the severity of exposure (see beyond). These observations point out two problems associated with large-scale application of these results. First, a pollution gradient analysis of animal population structure may not demonstrate the presumed effect of a pollutant or pollutants unless the gradient is steep (large difference in pollution levels at discrete distances from a point-source). Secondly, the results may be confounded by the presence of other mammals that may compete for the same resources required by the test population or species. Furthermore, demonstrable changes may require long periods of trapping, a factor that may not be compatible with available manpower and economic resources in wide-area studies. These problems are likely to constrain field research efforts unless accuracy and definitive results are sought.

Indirect Impact of Sulfur Dioxide at Different Trophic Levels

The interface between atmospheric gaseous pollutants and air-breathing vertebrates is the respiratory tract comprising nasal passageways, trachea, bronchi, and finally terminal bronchioles, alveolar sacs, and alveoli of the lungs. As SO_2 is probably the most toxic of the gaseous emissions from coal-fired power plants (other emission forms include particulates and flyash), its effect on lung tissue and subsequent animal health before and after atmospheric transformation of SO_2 is of considerable interest to students of ecological impact studies. The sulfur in coal is oxidized and released into the atmosphere. The majority of this sulfur is in the form of sulfur dioxide and the remainder (ca. 2%) is predominantly sulfuric acid (Rall, 1974). All SO_2 is not necessarily harmful. Sulfur is an essential growth element for plants, and sulfur deficient soils (as in the coast range of California) must be supplied with it. In high concentrations, the effects of SO_2 become noxious as decades of research and observations have shown. The sulfate radical is not toxic but sulfuric acid (or ammonium sulfate) can, in excess, build up soil acidity, with resultant loss of cations such as calcium which secondarily affects plant-forest productivity (Ovington, 1962). Indirectly, this deficiency could reduce the forage quality for both primary and secondary consumers in areas where cation stores are limited. Specific soil changes are complex and escape generalization since they are linked to soil acidity, total exchangable cations, soil differentiation, etc. (Tamm, 1977). Furthermore, all chemical changes may not be necessarily detrimental (Wood and Bormann, 1977).

Physiological Impact of Sulfur Dioxide on Vertebrates

Sulfur dioxide is highly soluble in the aqueous surface layer of the respiratory tree, and is consequently rapidly removed from the upper respiratory passages. In low concentrations, it is a mild respiratory irritant (Stokinger and Coffin, 1968). Characteristic signs of SO_2 injury include bronchial narrowing or constriction, arrested or altered ciliary beat, and inflammation of the mucous membranes (Frank and Speizer, 1965; Nadal, 1968; Talmage, 1977). Functionally, these adverse symptoms denote increased airway resistance, decreased effectiveness of lung clearance mechanisms, and epithelial cell and submucosal tissue damage. The extra-pulmonary effects of SO_2 are virtually unknown. Ocular and nasal irritations also occur in higher concentrations (10 ppm or greater)(Giddins and Fairchild, 1972). Deeper penetration of sulfur dioxide does not occur unless it is in particulate form, as for example the absorption of SO_2 of $SO_4^=$ onto the surface of an aerosol (*e.g.*, fog), which is 20 μm or less in diameter. Particulates 3 μm or less are especially effective, because at this size about 80% of the particulates entering the human lung are deposited in the alveolar ducts and sacs (Samfield, 1977). It is only when SO_2 penetrates to these terminal respiratory surfaces that lung function is affected (Rall, 1974). Irritation in tracheobronchial areas may produce discomfort but not necessarily lung pathologies or respiratory dysfunction. Guinea pigs exposed for 12 months to SO_2 concentrations up to 5.72 ppm showed no alterations in pulmonary function in several standard tests (Alarie *et al.*, 1970). Similarly, female beagles exposed to sulfur oxides demonstrated no changes in several hematological variables, while sulfuric acid applied at levels of about 900 μg/m^3 produced significant changes in several pulmonary characteristics, including lung volume and carbon monoxide diffusion capacity in addition to altered lung and heart weights (Lewis *et al.*, 1973). These results imply that sulfuric acid is far more toxic to pulmonary function than is SO_2 gas. Of particular interest at this point are the results of Amdur and Underhill (1970) who showed that openhearth dust at concentrations of 2.45 ppm or iron oxide (1.0 to 23 mg/m^3), both in aerosol form (< 0.3 μm diam), produced no detectable response in guinea pig respiration or when combined with several levels (1.5 to 26 ppm) of SO_2. These observations as well as those of others indicate that the SO_2 even in the presence of particulates may exert no detectable effects unless the SO_2 can be oxidized to sulfuric acid. Soluble metallic salts (e.g., manganese, ferrous iron, vanadium,) can potentiate the effects of SO_2 by catalyzing the oxidation of SO_2, whereas flyash or carbon particulates have no such potentiating effect (Amdur and Underhill, 1968). Thus, sulfur dioxide *per se* may not be an acute pulmonary health hazard until it is transformed into a more toxic form or is in sufficiently high levels to produce its irritating effects. These and other findings are discussed in depth in a recent document (Subcommittee, 1977).

Proposed Effects of SO_2 Upon ZAPS Small Mammals

Based upon the above brief discussion, one can examine the results of the ZAPS study in the light of two theoretical viewpoints. Firstly, deer mice may respond to SO_2 because of its irritant nature; it may produce multiple discomforts without affecting pulmonary function. Depletion of deer mice from a polluted area may result from avoidance behavior accentuated by the presence of particulates of appropriate size, density, shape and chemical composition, and which are capable of oxidizing SO_2.

Secondly, while the irritation produced by SO_2 (inter alia) may not constitute a direct health problem for mice at the relatively low concentrations produced by some power plants, it may nevertheless place a susceptible animal at a competitive disadvantage if an animal's survival depends on its frequent ability to maintain silence uninterrupted by coughing or sneezing and thereby alerting a nearby predator. A similar case may be developed for a pregnant animal that aborts when irritating fumes produce strong sensations to the olfactory receptors. More generally, the effect of a sublethal pollutant can be effective if it produces reactive behaviors in an animal that jeopardize its survival or that of its progeny or indirectly modifies steady-state processes for a sufficient period to elicit declines in animal performance.

What seems clear from these ideas is that to accurately assess the impact of a candidate pollutant requires more than the knowledge of its concentration and fraction of it reaching the organism. Depth of penetration, clearance mechanisms, and interaction with other pollutants or particulate emissions are critical in establishing the severity of inhalation exposure to power plant emissions. Gaps in our knowledge of these processes have been voiced recently in detail (Stuart, 1976).

Biological Contributions of Small Mammals

Small mammals (mice, voles, shrews, squirrels, etc.) are widely distributed across the North American continent, and affect the soil, vegetation, and the predators who eat them. These interactions are exceedingly complex. Heavy grazing (as in a population high) may impair not only the standing crop but eliminate a plant species as well. If less severe, grazing may stimulate new growth (Goszcyǹska and Goszcyǹski, 1977). Plant consumption helps to short circuit the mineralization process of organic material by virtue of the high rate of energy transformation in small mammals (Golley *et al.*, 1975). Seed consumption may favor seed dispersal. Surface activity, especially the establishment of microtine trails, can be extensive, allowing the growth of exotic species and producing a vegetational mosaic (Batzli, 1975). Burrows favor soil water retention and excretory products may contribute to soil fertility. In the Colstrip area, deer mice are the most abundant of the small mammals. Their populations are relatively stable and exert little grazing effects in this area dominated by grazing cattle, which probably affect the vegetational quality and distribution more than all small rodents combined. Deer mice are facultatively insectivorous and may exert some degree (probably small) of insect control. In brief, small mammals of the grasslands are valuable resources and probably do no harm to the landscape.

CONCLUSIONS

Overnight live trapping of ZAPS plots in 1976 and 1977 showed *Microtus ochrogaster* and *Peromuscus maniculatus* as the most abundant small mammals, about 74% of them *P. maniculatus*. Greater numbers of both species existed at ZAPS II, although animal biomass increased throughout late summer at both ZAPS plots. The colony-forming voles showed non-uniform distributions that did not appear to change with time. No conclusions could be drawn as to their sensitivity to SO_2. On the other hand, the ubiquitous deer mouse showed several distinct patterns of movement or trapping indicating SO_2 sensitivity. These patterns included larger numbers of adult mice on control plots, especially in mid to late summer; larger numbers of juvenile transients on plots A and B, compared to plots C and D, especially at ZAPS II; and substantially greater animal activity within control plot A, compared to plots B, C, and D. Despite these observations, the population effects of exposure to coal-fired power plant emissions cannot yet be fully evaluated. However, this study indicates that small mammal populations may be affected by long term exposure to relatively low SO_2 levels.

ACKNOWLEDGEMENTS

I am indebted to several individuals who patiently assisted me in the collection of animal data: T. Gullett, J. Rothermel, J. M. Chilgren, J. Unwin, and A. McPherson. In addition I have appreciated the encouragement afforded by R. A. Lewis, M. L. Morton, and E. Preston.

REFERENCES

Alarie, Y., C. E. Ulrich, W. M. Busey, H. E. Swann, Jr., and H. N. McFarland. 1970. Long-Term Continuous Exposure of Guinea Pigs to Sulfur Dioxide. Arch. Environ. Health, 21(6):769-777.

Ambrose, H. W. 1969. A Comparison of *Microtus pennsylvanicus* Home Ranges as Determined by Isotope and Live Trap Methods. Amer. Midl. Nat., 81:535-555.

Amdur, M. O. and D. W. Underhill. 1968. The Effect of Various Aerosols on the Response of Guinea Pigs to Sulfur Dioxide. Arch. Environ. Health, 16:460-468.

Amdur, M. O. and D. W. Underhill. 1970. Response of Guinea Pigs to a Combination of Sulfur Dioxide and Open Hearth Dust. J. Amer. Poll. Cont. Assoc., 20:31-34.

Batzli, G. O. 1975. The Role of Small Mammals in Arctic Ecosystems. In: Small Mammals: Their Productivity and Population Dynamics, F. B. Golley, K. Petrusewicz, and L. Ryszkowski, eds. Cambridge University Press, N.Y. pp. 243-268.

Brown, L. E. 1966. Home Range and Movement of Small Mammals. Proc. Zool. Soc. London, 18:111-142.

Davis, D. E. 1964. Manual for Analysis of Rodent Populations. University Microfilms, Ann Arbor, Michigan. 82 pp.

Delaney, M. J. 1975. The Ecology of Small Mammals. Crane, Russak & Co., N.Y. 60 pp.

Eadie, W. R. 1953. Response of *Microtus* to Vegetative Cover. J. Mammal., 34:263-264.

Frank, N. R. and F. E. Speizer. 1965. SO_2 Effects on the Respiratory System in Dogs: Changes in Mechanical Behavior at Different Levels of the Respiratory System During Acute Exposure to the Gas. Arch. Environ. Health, 11:624-634.

French, N. R., D. M. Stoddart, and B. Bobek. 1975. Patterns of Demography in Small Mammal Populations. In: Small Mammals: Their Productivity and Population Dynamics, F. B. Golley, K. Petrusewicz, and L. Ryszkowski, eds. Cambridge University Press, N.Y. pp. 73-102.

Giddens, W. E. and G. A. Fairchild. 1972. Effects of Sulfur Dioxide on the Nasal Mucosa of Mice. Arch. Environ. Health, 25:166-173.

Golley, F. B., L. Ryszkowski, and J. T. Sokur. 1975. The Role of Small Mammals in Temperate Forests, Grasslands and Cultivated Fields. In: Small Mammals: Their Productivity and Population Dynamics, F. B. Golley, K. Petrusewicz, and L. Ryszkowski, eds. Cambridge University Press, N.Y. pp. 223-242.

Goszczyńska, W. and J. Goszczyński. 1977. Effect of the Burrowing Activities of the Common Vole and the Mole on the Soil and Vegetation of the Biocenoses of Cultivated Fields. Acta Theriol., 22:181-190.

Harvey, M. J. and R. W. Barbour. 1965. Home Range of *Microtus ochrogaster* as Determined by a Modified Minimum Area Method. J. Mammal., 46:398-405.

Lewis, T. R.,W. J. Moorman, W. F. Ludmann, and K. I. Campbell. 1973. Toxicity of Long-Term Exposure to Oxides of Sulfur. Arch. Environ. Health, 26:16-21.

Metzgar, L. H. 1973. Exploratory and Feeding Home Ranges in *Peromyscus*. J. Mammal., 54(3):760-763.

Morris, R. D. 1970. The Effects of Endrin on *Microtus* and *Peromyscus*. I. Unenclosed Field Populations. Can. J. Zool., 28:695-708.

Nadal, J. A. 1968. Mechanisms of Airway Response to Inhaled Substances. Arch. Environ. Health, 16:171-174.

Ovington, J. D. 1962. Quantitative Ecology and the Woodland Ecosystem Concept. Adv. Ecol. Res., 1:103-192.

Rall, D. P. 1974. Review of the Health Effects of Sulfur Dioxide. Environ. Health Perspect., 8:97-121.

Samfield, M. 1977. Hazardous and Toxic Air Pollutants. Energy Sources, 3(2):111-124.

Stickel, L. F. 1960. *Peromyscus* Ranges at High and Low Population Densities. J. Mammal., 41:433-441.

Stickel, L. F. 1968. Home Range. In: Biology of *Peromyscus* (Rodentia), J. A. King, ed. Sp. Publ. No. 2. Amer. Soc. Mammalogists. pp. 373-411.

Stokinger, N. E. and D. L. Coffin. 1968. Biologic Effects of Air Pollution. In: Air Pollution, A. C. Stem, ed. Academic Press, N.Y. pp. 445-546.

Stuart, B. O. 1976. Deposition and Clearance of Inhaled Particles. Environ. Health Perspect., 16:41-53.

Subcommittee on Airborne Particles, Committee on Medical and Biologic Effects of Environmental Pollutants. National Research Council, NAS. 1977. Airborne Particles. EPA-600/1-77-053. Research Triangle Park, North Carolina. 555 pp.

Talmage, S. S. 1977. Humans: Metabolism and Biological Effects. In: Environmental, Health, and Control Aspects of Coal Conversion: An Information Overview, H. M. Baaunstein, E. D. Copenhaver, and H. A. Pfuderer, eds. ORNL/EIS-95, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Ch. 10, Vol. 2, 99 pp.

Tamm, C. O. 1977. Acid Precipitation and Forest Soils. Water, Air, and Soil Pollution, 7:367-370.

Taylor, J. E. and W. C. Leininger. 1978. Monitoring Plant Community Changes Due to SO_2 Exposure. In: The Bioenvironmental Impact of a Coal-fired Power Plant, E. M. Preston and R. A. Lewis, eds. 3rd Interim Report, Colstrip, Montana. EPA-600/3-78-021. Environmental Protection Agency, Corvallis, Oregon. pp. 376-384.

Terman, C. R. 1968. Population Dynamics. In: Biology of *Peromyscus* (Rodentia), J. A. King, ed. Sp. Publ. No. 2. Amer. Soc. Mammalogists. pp. 412-449.

Wood, T. and F. H. Bormann. 1977. Short-term Effects of a Simulated Acid Rain Upon the Growth and Nutrient Relations of *Pinus strobus*, L. Water, Air, and Soil Pollution, 7:479-488.

AN EXPERIMENTAL EVALUATION OF THE FATE AND IMPACT OF SELECTED TRACE ELEMENT STACK EMISSIONS IN THE SOIL-PLANT ENVIRONMENT

ing page blank

SECTION 21

THE FATE AND BIOENVIRONMENTAL IMPACT OF MERCURY IN SOILS

E. R. Landa

ABSTRACT

Five surface soils from southeastern Montana were utilized in these studies. The volatile loss of mercury applied to these soils as mercuric nitrate and methylmercury chloride was stimulated by elevated temperatures and repressed by excessive moisture or dryness. The addition of glucose stimulated the volatile loss of mercury in the case of mercuric nitrate, but repressed it in the case of methylmercury chloride. Mercury sorbed by soils previously exposed to elemental mercury vapor was shown to volatilize at 100-200 C, and to probably exist as a soil organo-complex. Short-term respiration studies involving soil amendment with mercuric chloride showed mercury levels greater than 40 μg/g were required to inhibit carbon mineralization. Longer-term respiration studies showed inhibition at levels from 0.1 to greater than 100 μg Hg/g depending on the soil type. Western wheatgrass seedlings grown on soils amended with mercuric nitrate showed aerial tissue concentration factors of 0.01 to 0.12.

INTRODUCTION

With the development of the energy resources of the western United States have come concerns regarding the environmental impact of the emissions associated with power-generation on the grassland biome. While coal from the Powder River Basin of northeastern Wyoming and southeastern Montana contains an average of only 0.09 ppm Hg (Swanson *et al.*, 1974), the scrubbers and electrostatic precipitators used to control air pollution from coal-fired power plants are ineffective in the removal of Hg, and about 90% of that in the coal is released to the environment (Billings and Matson, 1972; Montana State Department of Natural Resources and Conservation, 1974). In addition to the vast coal resources of the western states, there exists numerous sites for the potential development of geothermally-powered electric generating plants. Mercury present in such geothermal reservoirs can be released to the surface environment with the live stream during drilling and venting, and from the spent steam in condensate ponds (U.S. Dept. of Interior, 1973; Siegel and Siegel, 1975).

The toxicity of mercury to man has been recognized since the 16th century (D'Itri, 1972). When operating at full capacity, the stacks of Colstrip Units 3 and 4 (700 MW each) will each emit an estimated 55 grams of mercury per hour (Montana State Department of Natural Resources and Conservation, 1974). The chemical nature of the mercury emitted has not been determined, but Hg^o and Hg^{2+} appear to be likely candidates. In the environment, either of these forms is subject to a variety of transformations, including methylation (D'Itri, 1972).

The major foci of the soils research on this project have been study of:

(1) the volatile loss of soil-applied inorganic and organic mercury,
(2) the retention of metallic mercury vapor by soils,
(3) the uptake from soil of divalent inorganic mercury by plants,
(4) the effect of divalent inorganic mercury on soil microbial respiration.

The Volatile Loss of Soil-Applied Inorganic Divalent Mercury

Mercury is an environmentally mobile element, cycling through the lithosphere, atmosphere and hydrosphere. The volatile loss of applied inorganic divalent mercury from soils was first reported by Zimmerman and Crocker (1933, 1934) who observed foliar damage in plants grown near soils treated with either mercuric bromide, chloride, cyanide, iodide, nitrate, oxide or sulfate. The damage was similar to that seen with exposure to metallic mercury vapor, and indeed the presence of this species was demonstrated in the atmosphere above soil treated with $HgCl_2$. Several recent investigations (Gracey and Stewart, 1974; Johnson and Braman, 1974) have quantified the loss of mercury from soils amended with inorganic mercuric salts.

Alberts *et al.* (1974) showed that the loss of Hg from $Hg(NO_3)_2$ -humic acid suspensions was as metallic mercury, Hg^o, and that the process was probably abiotic. DeFilippis and Pallaghy (1975) showed ethylene and acetylene capable of non-enzymatically reducing mercuric-Hg, in aqueous solution as $HgCl_2$, to a volatile form, presumably Hg^o. However, volatilization of Hg by a variety of microorganisms *in vitro* has also been demonstrated. Thayer (1976), and Ben-Bassat and Mayer (1975) reported the loss of Hg from $HgCl_2$-amended cultures of the alga *Euglena gracilisis* and *Chlorella pyrenoidosa* respectively. Brunker and Bott (1974) showed a yeast of the genus *Cryptococcus* capable of reducing Hg(II) to its elemental state. Sayler *et al.* (1975) isolated a species of *Pseudomonas* from Chesapeake Bay sediments capable of reducing Hg(II) to volatile Hg^o.

Frear and Dills (1967), using mortality in insect eggs exposed to the atmosphere above an Hg(II)-amended Hagerstown silt loam as an index of volatile mercury loss, found losses to increase with temperature from 1 to 25 C, and with soil moisture from 1 to 18%.

The Volatile Loss of Mercury From Soils Amended With Methylmercury Chloride

Due to the ability of methylmercury to cross the blood-brain barrier and cause irreversible brain damage in mammals (*i.e.* Minamata disease), its formation in soils and aquatic sediments subject to mercury amendment by industrial discharges or the agricultural usage of mercurials has been an area of considerable concern and investigation.

In studies on methylmercury synthesis, several investigators have reported the apparent loss of this compound from sediment and soil systems. Laboratory studies of aerated lake sediment-water systems by Spangler *et al.* (1973a) showed a build-up in methylmercury during the first 50 days of incubation with mercuric chloride, followed by a rapid decrease in the quantity of methylmercury in the system. River sediments treated with mercuric chloride (Jacobs and Keeney, 1974; Van Fraasen, 1975; Olson and Cooper, 1976) or phenylmercuric acetate (Jacobs and Keeney, 1974), and soils treated with mercuric nitrate (Rogers, 1976) showed an apparent decrease in methylmercury concentration with time. Kimura and Miller (1964) showed mercury losses of 6-14% to occur over a period of 35 days from a moist Puyallup sandy loam soil amended to about 80 ppm Hg applied as methylmercury dicyandiamide or methylmercury chloride.

The work reported here focuses on the roles of microbial activity, temperature and moisture on the volatile loss of mercury from soils amended with methylmercury chloride.

The Retention of Metallic Mercury Vapor by Soils

The sorption of metallic mercury vapor (Hg^o) by soils is of interest to geochemists concerned with the genesis of mercury ore bodies and with the environmental fate of atmospheric pollutants. Mercury may be introduced into the atmosphere from natural sources including zones of active volcanism and mercury mineralization, and by a range of human activities including ore refining and coal combustion.

The maximum mercury concentration measured by the U.S. Geological Survey (McCarthy *et al.*, 1970) in the air over unmineralized, non-industrial areas of the western United States ranged from 0.003 to 0.009 $\mu g/m^3$. A survey by Jepsen (1973) of elemental mercury vapor concentrations in the air of selected U.S. urban areas showed levels up to 4 $\mu g\ Hg^o/m^3$. The mercury vapor concentration of the air above fumaroles in Hawaii has been reported to be about 22 $\mu g/m^3$ (Eshleman *et al.*, 1971), while concentrations as high as 2000 $\mu g/m^3$ have been reported for the air in mercury mines (McCarthy *et al.*, 1970). Mercury vapor concentrations of 2 to 31 $\mu g/m^3$ have been measured in flue-gas resulting from the combustion of coals containing 0.15 to 0.3 ppm Hg (Billings and Matson, 1972; Diehl *et al.*, 1972). The threshold limit value for occupational exposure to mercury vapor in air has been set at 10 and 100 $\mu g/m^3$ in the U.S.S.R. and the U.S.A. respectively (Schroeder, 1970).

Metallic mercury vapor (Hg^o) was reported as the dominant Hg species in the near-ground atmosphere of the Tampa Bay, Florida area (Johnson and

Braman, 1974), and in the incoming steam and gaseous effluents at two geothermal power plants in California and Mexico (Robertson *et al.*, 1977). Piperno (1975) reported mercury to occur principally in the elemental form in coal combustion emissions.

The retention of Hg^o vapor by clays and organic matter (Koksoy and Bradshaw, 1969) has been postulated as causative factors in the occurrence of mercury anomalies ("haloes") surrounding ore bodies. Trost and Bisque (1972) examined the sorption of Hg^o by soil components, and found much greater sorption (per unit weight) by organic materials as compared to clay minerals. The objective of the work reported here was to examine the sorption of Hg^o by a range of surface soils.

The Effect of Mercuric Chloride on Carbon Mineralization in Soils*

The fungicidal and bactericidal properties of mercurial compounds has long been recognized and utilized in agriculture and medicine. However, little attention has focused upon the quantitative assessment of the impact of inorganic mercuric compounds on soil microorganisms in situ.

Mercuric ion toxicity presumably is a consequence of the displacement of the normal suite of catalytic metal ions from their binding sites on a protein, particularly the sulfhydryl groups, and the resultant impairment of enzyme function (Hughes, 1972). Two common soil fungi, *Aspergillus niger* and *Penicillium notatum*, were found to grow normally in liquid medium containing up to 10 ppm Hg as $HgCl_2$ (Hardcastle and Mavichakana, 1974). Oxygen-consumption by baker's yeast (*Saccharomyces cerevisiae*) grown in liquid culture was severelv limited at 100 ppm $HgCl_2$ (Kokke, 1974). Tonomura *et al.* (1968) reported on a mercury-resistant, soil-isolated *Pseudomonas* whose growth was uninhibited at $HgCl_2$ levels in solution culture of below 450 ppm. Van Faassen (1973) showed 100 ppm $HgCl_2$ amendment of two soils (one of which had a history of treatment with mercurial fungicides) to have little effect on the number of microbes plated, but to reduce carbon and nitrogen mineralization and dehydrogenese activity. Peterson (1962) showed that moistening of a silty clay loam soil to 87 percent of its water holding capacity with either 0.001 or 0.01 M $HgCl_2$ reduced its oxygen consumption in 50 hours by 24 or 80%, respectively.

This study was designed to assess the short- and long-term effects of mercuric chloride amendments to a variety of soils on CO_2-evolution from glucose and native soil carbon substrates.

The Uptake From Soils of Inorganic Divalent Mercury by Plants

With the release of mercury to the grassland ecosystem of the Northern Great Plains by coal combustion and geothermal energy exploitation comes the potential for bioaccumulation of mercury. Western wheatgrass (*Agropyron smithii*) is a widely distributed range species in the western United States and Canada, and is often the dominant species in the Northern Great Plains (Martin, 1969). Little is known regarding its uptake of heavy metals, a computerized search of Biological Abstracts since 1972 revealing only a single study (Munshower and Behan, 1971). The uptake of mercury from soils by western wheatgrass was therefore studied in a greenhouse experiment.

---

*Work done jointly with S. C. Fang.

MATERIALS AND METHODS

The five surface soils (0-20 cm depth) utilized in these studies were collected in May, 1976 from uncultivated sites in Treasure and Powder River Counties, Montana. The soils were air-dried and ground to pass a 2 mm sieve. The soil pH (1:2 soil:water), calcium carbonate equivalent, organic carbon (Walkley-Black titration), total Kjeldahl nitrogen, extractable P (0.5 *M* sodium bicarbonate, pH 8.5), total soluble salts (saturation extract electrical conductivity), extractable potassium and sodium, and cation exchange capacity (1*N* ammonium acetate, pH 7) were determined (Kauffman and Gardner, 1976). Native soil mercury levels were determined by flameless atomic absorption spectrometry following digestion of the soil with aqua regia. Particle size analysis was by the pipette method (Day, 1965). The 1/3-bar and saturation moisture percentages was determined by the method of the U.S. Salinity Laboratory Staff (1954). The results of these analyses are presented in Tables 21.1 and 21.2.

The Volatile Loss of Soil-Applied Inorganic Divalent Mercury

Microbial Aspects of the Volatile Loss of Soil Applied Inorganic Hg (II)

Prior to Hg(II) amendment, the soils were either untreated, autoclaved at 121 C for 1 hour, incorporated with glucose at a 1% (w/w) rate, or incorporated with glucose (1% w/w) and KNO_3 (0.3% w/w).

Three replicate samples of each soil, 70 g (oven-dry basis) were amended to 1 μg Hg/g soil using $^{203}Hg\text{-}Hg(NO_3)_2$ applied with sufficient water to reach the 1/3-bar moisture content. The amended soils, contained in 150 ml plastic cups, were covered with 40 g of coarse quartz sand, watered every other day to the 1/3-bar moisture level and maintained at room temperature (18-25 C) in a forced-draft fume hood.

The loss of Hg from the soils was monitored by radioassay of the entire soil mass using a small animal, whole-body, liquid scintillation spectrometer system (Armac model 446, Packard Instrument Co.). A special sample holder established a reproducible counting geometry in the sample well. The samples were counted, using the 0.28 MeV γ-radiation emitted by ^{203}Hg, immediately after soil amendment, every other day for the first 10 days, and at weekly intervals for the next 4 weeks.

To determine if any Hg volatilization process might be reactivated after 38 days, the autoclaved soils were irrigated with an inoculum solution (supernatant of an incubated and settled 2% w/w soil:water slurry) to reintroduce the indigenous microflora. The glucose-, and glucose plus KNO_3-treated soils were irrigated with 1% solutions of the respective substrates. The moisture content of these samples was maintained at the 1/3-bar level using the inoculum, glucose or glucose plus KNO_3 solutions rather than distilled water. These samples were monitored for an additional 2 weeks.

TABLE 21.1. PHYSICAL PROPERTIES OF SELECTED EASTERN MONTANA SOILS

Soil Series	Classification	Sand %	Silt %	Clay %	Water Holding Capacity, % 1/3-bar	Saturation
Arvada	Ustollic Natrargid, fine, montmorillonitic, mesic	35	25	40	31	54
Campspass	Typic Eutroboralf fine, montmorillonitic	19	56	25	33	80
Heldt	Usteric Camborthid, fine, montmorillonitic, mesic	27	48	25	21	52
Bainville	Ustic Torriorthent, fine, silty, mixed (calcareous), mesic	29	42	29	21	45
Terry	Ustollic Haplargid, coarse-loamy, mixed, mesic	74	14	12	10	37

TABLE 21.2. CHEMICAL PROPERTIES OF SELECTED EASTERN MONTANA SOILS

Soil Series	pH	$CaCO_3$ equiv. %	Organic C, %	Total N, %	Extractable P ppm	K ppm	Na meq/100g	Total Soluble Salts mmho/cm	Cation Exchange Capacity meq/100g	Total Hg μg/kg
Arvada	8.1	0.08	1.6	0.10	3	526	0.87	0.43	22.2	<60
Campspass	6.6	—	6.7	0.27	5	203	0.07	0.35	25.5	130
Heldt	8.3	3.92	1.7	0.12	1	186	0.08	0.35	11.4	183
Bainville	7.5	0.04	1.0	0.08	2	231	0.07	0.28	15.8	73
Terry	8.3	0.20	0.9	0.10	1	84	0.07	0.31	8.4	<60

Soil Water Content and Soil Temperature as Factors in the Volatile Loss of Soil-Applied Inorganic Hg (II)

The soils, in 70 g (oven-dry basis) portions, were amended to 1 μg Hg/g soil using ^{203}Hg-Hg$(NO_3)_2$ applied with sufficient water to reach the 1/3-bar or 80% saturation moisture content. The amended soils, contained in 150 ml plastic cups, were covered with 40 g of coarse quartz sand, and watered every other day to maintain the desired moisture level. The moisture contents maintained at room temperature (18-25 C) were 80% saturated or 1/3-bar. A third set of samples was initially moistened to the 1/3-bar level, then allowed to air-dry; after 25 days the soils were re-moistened to the 1/3-bar level. The temperature studies were run at room temperature, 10 or 35 C, the latter two temperatures controlled by immersion of the cups in a water bath, and involved samples maintained at the 1/3-bar moisture content. There were 3 replicates per soil in each experiment and all samples were stored in a forced-draft fume hood.

The loss of Hg from the soils was monitored by radioassay of the entire soil mass as described above. After 38 days, the samples equilibrating at 10 C were brought to room temperature and monitored for an additional 2 weeks.

The Volatile Loss of Mercury From Soils Amended with Methylmercury Chloride

In order to examine the role of the microbial population in the volatilization process, the soils were either untreated, autoclaved at 121 C for 1 hour, or supplemented with glucose (1% w/w) and KNO_3 (0.3% w/w) prior to mercury amendment. Three replicate samples of each soil (70 g, oven-dry basis) were amended to 1.0 μg Hg/g soil using methylmercury chloride, ^{203}Hg-CH_3HgCl, applied with sufficient water to reach the 1/3-bar moisture content. The amended soils, contained in 150 ml plastic cups, were covered with 40 g of coarse quartz sand, watered every other day to the 1/3-bar moisture level and maintained at room temperature (20-27 C) in a forced-draft fume hood.

In order to examine the influence of soil moisture content and temperature on the volatilization process, soils in 70 g portions were similarly-amended to 1.0 μg Hg/g soil with methylmercury chloride applied with sufficient water to reach either the 1/3-bar or 80% of saturation moisture content, and were maintained at room temperature at these respective moisture contents by alternate-day distilled water additions. A third set of samples was initially moistened to the 1/3-bar level, then allowed to air-dry; after 28 days these soils were re-moistened to the 1/3-bar level. The temperature studies were run at room temperature (20-27 C), or controlled at 16 or 35 C by immersion of the cups in a water bath. Samples were maintained at the 1/3-bar moisture content, replicated 3 times and were kept in a forced-draft fume hood.

The loss of Hg from the soils was monitored by radioassay of the entire soil mass as described above. The samples were counted using the 0.28 MeV γ-radiation of the ^{203}Hg immediately after soil amendment, every-other day for the first 12-18 days and at weekly intervals for the next 5-6 weeks.

The Retention of Metallic Mercury Vapor by Soils

Forty gram (oven-dry basis) portions of air-dried soil were placed in 100 mm diameter polystyrene petri dishes and stacked in a 23 liter glass bell jar (Figure 21.1). There were 3 exposure runs and 10 plates of each of the 5 soils per run (2000 g of soil per run). Mercury vapor was generated from a ^{203}Hg-labelled metallic mercury source having an initial specific activity of 60 mCi/g which was contained in a thermoregulated water bath to control vapor concentration. Air was passed over this metallic mercury and down a glass inlet tube to the bottom of the bell jar which contained the soil samples. The petri dishes of each soil were arranged randomly in a 13-tier support rack within the bell jar. The air exited near the top of the bell jar with the flow rate maintained at 200 ml/min. A magnetically-driven fan at the base inside the bell jar mixed the air within the jar such that the outlet vapor concentration of Hg was assumed to be the ambient concentration (Slatyer, 1971). The internal surfaces of the bell jar and the petri dish support rack were lightly coated with petroleum jelly to minimize the sorption of mercury vapor.

Air exiting the bell jar passed through a series of traps containing Hopcalite, a granular copper-manganese oxide material (Magos, 1966), to trap the mercury vapor. The traps were radioassayed on alternate days using a NaI γ-scintillation detector. The soils were exposed to air containing an average outlet concentration of 14.3 μg Hg/m^3 for 10 days and then purged for 2 days with room air. During the exposure and purging periods, the soils were at room temperature (22-34 C).

At the end of each purge period, the plates of each of the five soils were radioassayed using a small animal, whole-body, liquid scintillation spectrometer system (Armac model 446, Packard Instrument Co.) and then the individual soils were bulked and mixed.

To assess the volatile loss of the retained mercury vapor from these soils, 60 g (oven-dry basis) samples of soil covered with 40 g of coarse quartz sand and maintained at room temperature either air-dry or at the 1/3-bar moisture content (by alternate day additions of distilled water) in small plastic cups kept in a forced draft fume hood, were monitored for 16 days using the Armac system.

To assess the heat lability of the retained mercury, 1.5 g samples of soil contained in 2 ml shell vials were heated in an oven for 2 days to temperatures ranging from 80 to 450°C. A fresh sample was used for each temperature, and there were 3 replicates per soil per temperature. The fraction of the initially-retained mercury remaining following the heat treatment was determined by radioassay of the vials using a NaI γ-scintillation spectrometer (Packard Auto-Gamma Spectrometer Model 5230).

To assess the extractability of the mercury by various chemical agents, 10 g (oven dry basis) of each soil in triplicate were extracted by overnight shaking at room temperature with 20 ml of the following solutions:

(a) distilled water
(b) 1*N* KCl

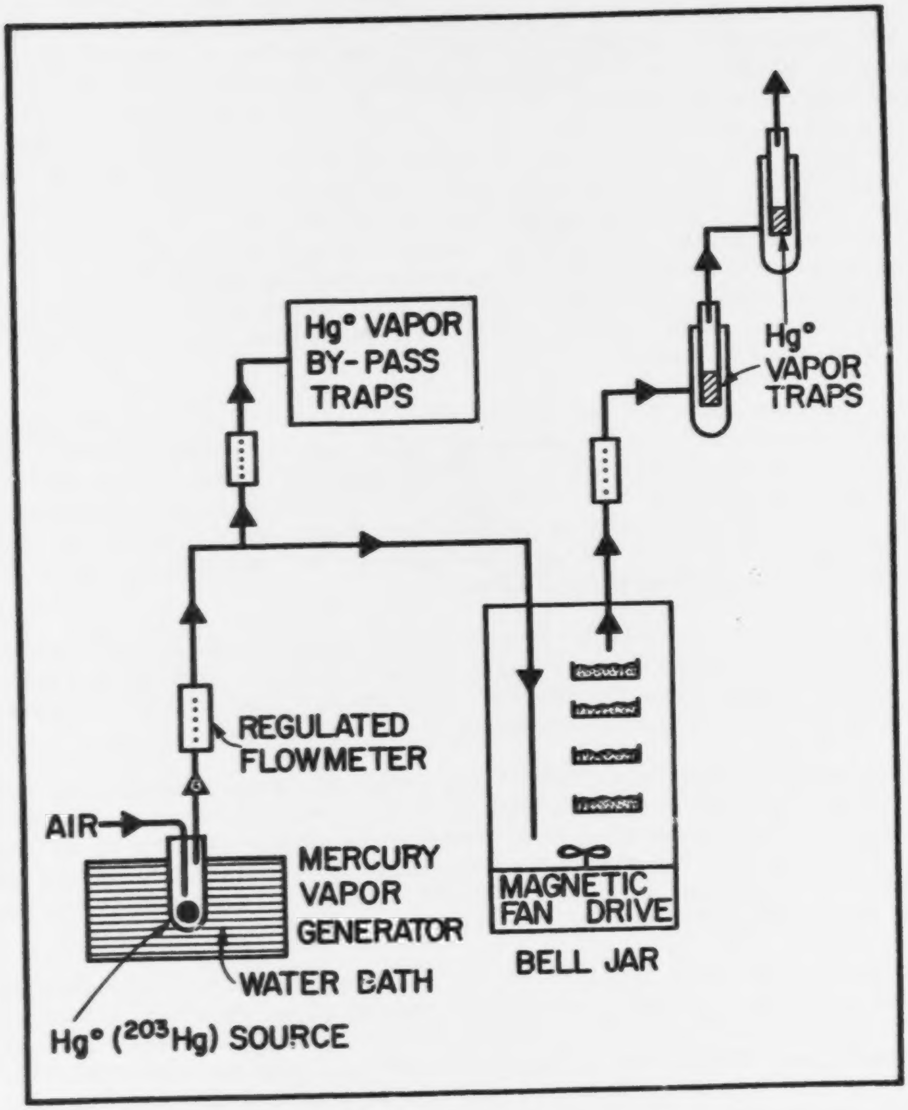

Hg° VAPOR
BY-PASS
TRAPS
Hg°
VAPOR
TRAPS
REGULATED
FLOWMETER
AIR
MERCURY
VAPOR
GENERATOR
WATER BATH
Hg° (^{203}Hg) SOURCE
MAGNETIC
FAN DRIVE
BELL JAR

Figure 21.1. Schematic representation of system used to expose soils to metallic mercury vapor.

(c) $1N$ $CaCl_2$
(d) $1N$ HCl
(e) $1N$ NaOH
(f) $0.5M$ acetylacetone (2, 4-pentanedione)
(g) $0.5M$ cupferron (the NH_4-salt of N-nitrosophenylhydroxylamine)
(h) $0.5N$ Cu $(C_2H_3O_2)_2$
(i) $.005M$ DTPA (diethylenetriamine pentaacetic acid): $0.01M$ triethanolamine: $0.1M$ $CaCl_2$; pH 7.3 solution.
(j) $0.1M$ cysteine (extraction run at 4 C to inhibit microbial activity)
(k) benzene
(l) methanol

After shaking, the tubes were centrifuged at 27,000 x G for 10 minutes, and 4 ml aliquots of the supernatant were sampled for radioassay using the NaI spectrometer.

The Effect of Mercuric Chloride on Carbon Mineralization in Soils

Short-term radiorespirometric studies utilized uniformly-labelled ^{14}C-glucose. Five grams of soil was transferred to each reaction flask containing 10 ml of water, or $HgCl_2$ solution (1×10^{-4} M to 1×10^{-3} M) spiked with 0.5 µCi (0.43 µg) of ^{14}C-glucose. The samples were maintained at 25 C for 6 hours and continually flushed with CO_2-free air. Respired $^{14}CO_2$ was trapped in 0.5 N NaOH, precipitated as $BaCO_3$, transferred to a weighed glass fiber filter disc and assayed with a windowless gas flow proportional counter. Correction was made for self-absorption by the $BaCO_3$ filter cake. Each treatment was replicated four times.

Long-term studies utilized no added carbon substrate. Bulk soil samples were moistened to 50% of the 1/3-bar moisture holding capacity with distilled water and incubated for one week at 25 C. The soils were amended to 0, 0.1, 1.0, 10, or 100 µg Hg (as $HgCl_2$)/g soil with sufficient water to bring them to the 1/3-bar moisture level. Twenty-five gram samples (oven-dry basis) of treated soil were placed in 1 quart mason jars containing a flask with 10 ml of dilute standardized NaOH. The jars were sealed, maintained at 25 C, and, at weekly intervals for a month, opened for reaeration and sampling of the NaOH traps. Carbonates were precipitated as $BaCO_3$ and the remaining alkali titrated with HCl as outlined by Stozky (1965). Each treatment was replicated three times.

The Uptake from Soils of Inorganic Divalent Mercury by Plants

The soils, in 70 g (oven-dry basis) portions were amended with 1.0 µg Hg/g soil using ^{203}Hg-$Hg(NO_3)_2$ applied with sufficient water to reach the 1/3-bar content, and contained in plastic cups without drainage holes. There were 3 replicates per soil per treatment. After a 24 hour equilibration period, twenty seeds of "Rosana" western wheatgrass were applied to the surface of the soil and covered with 40 g of moist sand. The plants were grown in a chemical fume hood located in a greenhouse. Distilled water was added on alternate days to make up for evapotranspiration losses and bring the soils back to the 1/3-bar moisture content. No fertility amendments were made to the soil either initially nor during the course of the experiment. Natural illumination was supplemented by incandescent lights (G.E.

Gro-Sho; 16 hour day length) whose heat was dissipated by a flowing-water filter cell. Twenty-two days after planting, the above-ground tissue was harvested, radioassayed using an NaI γ-scintillation spectrometer (Packard model 5230) and dried at 80 C. Tissue Hg concentrations were calculated from the specific activity of the added Hg assuming no dilution by the endogenous soil mercury pool.

In order to assess the extent of volatile loss of the added Hg from the soils during the course of plant growth, soil ^{203}Hg content was monitored by radioassay of the entire soil mass using a small animal, whole-body, liquid scintillation spectrometer system (Armac model 446, Packard Instrument Co.). A special sample holder established a reproducible counting geometry in the sample well. The samples were counted using the 0.28 MeV γ-radiation of the ^{203}Hg after soil amendment, and again after tissue harvest.

RESULTS AND DISCUSSION

The Volatile Loss of Soil-Applied Inorganic Divalent Mercury

Microbial Aspects of the Volatile Loss of Soil-Applied Inorganic Hg (II)

Figure 21.2 shows the effects of the treatments to suppress and to stimulate microbial activity on the volatilization of Hg from the soils examined. In general, autoclaving reduced the total loss, while glucose additions increased the initial loss rate of the applied Hg(II) from these soils. After 38 days, neither the addition of a microbial inoculum to the autoclaved soils, nor fresh additions of C or C + N substrate to the glucose- or glucose plus KNO_3-supplemented, non-sterile soils provide for any further Hg losses. With the exception of the Bainville soil, the addition of a nitrogen source to the glucose had little effect on the losses observed, suggesting that a C:N ratio imbalance was not, in general, involved in the termination of Hg loss. The coefficient of variation for these experiments averaged 1.3%.

As it was not possible to suppress microbial growth in the autoclaved soils after the start of the experiment, the Hg losses seen here may be the result of an abiotic volatilization process and/or a biologically-mediated process associated with the recolonization of the autoclaved soil (Baker, 1962). Other workers have reported soil sterilization to decrease the loss of Hg^{o} from soils amended with $HgCl_2$ (Rissanen, 1973), phenylmercuric acetate (PMA) and ethylmercuric acetate (Kimura and Miller, 1964), although in the case of PMA, the rate of loss after the first 2 weeks was in excess of that observed in non-sterile systems. Frear and Dills (1967) attribute the progressive decrease in the quantity of Hg^{o} evolution they observed with $HgCl_2$ amendments above 300 μg Hg/g soil to mass action effects. However, in the 300 to 2400 μg Hg/g soil range they examined, inhibition of microbial activity by Hg toxicity must certainly be considered a possible causal factor.

The marked acceleration of the Hg loss rate accompanying glucose incorporation, seen particularly in the Heldt and Terry soils, together with the suppression of loss associated with autoclaving, suggests a major role for the soil biota. Brunker and Bott (1974) showed the mercury content of an

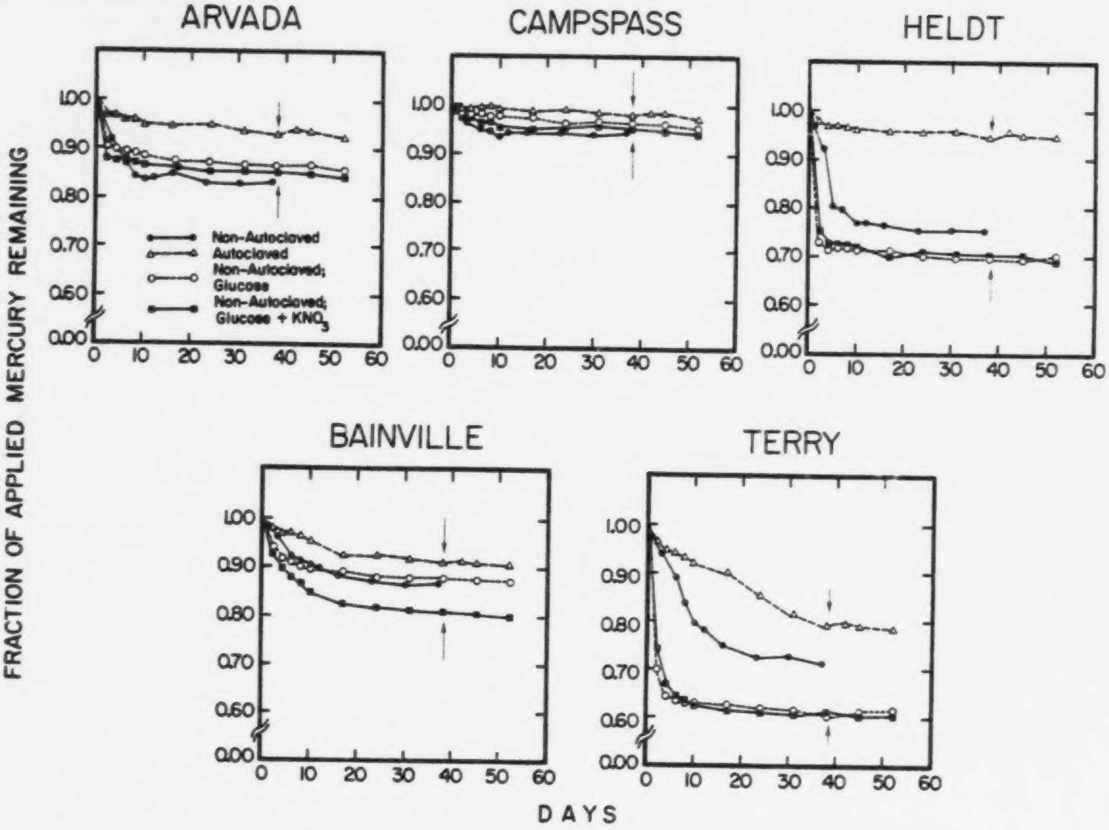


Figure 21.2. Effect of autoclaving, glucose, and glucose + KNO_3 on Hg loss from mercuric nitrate-amended soils. Vertical arrow at day 38 indicates initiation of irrigation with inoculum suspension or metabolic substrate solution.

$HgCl_2$-amended, sterile microbial medium containing 1% glucose to remain unchanged over a period of 17 days, suggesting that the presence of glucose in the absence of microorganisms does not promote the volatile loss of mercury from Hg(II)-amended systems. A burst of microbial activity is associated with the moistening of a non-sterile soil with glucose (Peterson, 1962). This stimulated metabolic activity presumably correlates with the rapid early losses observed. Microbial respiration studies involving these soils (Landa and Fang, 1978) showed the Heldt and Terry soils to have the lowest respiration rates, and the Campspass soil to have the highest. Thus the greater volatilization seen with the glucose additions to the former soils may reflect their relative lack of indigenous, readily-oxidizable substrates. The pattern is similar to that observed with the volatile loss of selenium from soils incorporated with readily-available metabolic substrates (Abu-Erreish *et al.*, 1968; Doran and Alexander, 1977).

Zimmerman and Crocker (1933) found more severe mercury vapor injury to roses grown in soils treated with both a high organic matter tankage fertilizer and $HgCl_2$, as compared to those grown on soils receiving $HgCl_2$ alone. This effect could be attributed to either a humic acid-mediated abiotic process such as shown by Alberts *et al.* (1974) or a microbial stimulation as demonstrated in this study. Van Faassen (1973) found that the addition of glucose to an $HgCl_2$-amended soil speeded the recovery of dehydrogenase activity. This enhanced restoration of microbial activity was perhaps associated with an accelerated removal of mercury from the soil catalyzed by the addition of glucose.

While the forms of mercury being lost from the soil were not identified, Hg^o seems likely to be the major species. Rogers (1975) showed Hg^o to be the predominant form lost from an $Hg(NO_3)_2$-amended calcareous fine sandy loam desert soil, with lesser amounts of methyl-, mercuric- and dimethyl-species being detected in the atmosphere above the soil. Johnson and Braman (1974) showed similar results for $HgCl_2$-amendment of a Florida turf soil. Van Faassen (1975) and Rogers (1976) found little methylation of applied Hg(II) in soils.

After 2-24 days, depending upon soil and treatment, little or no further Hg losses were observed. The remaining Hg appeared to have "stabilized" after losses of from 5-40% of the initial amendment. In general, for the non-autoclaved soils, the amount of Hg-loss followed the order:

Terry>Heldt>Bainville, Arvada>Campspass

Frear and Dills (1967) found a similar decrease in mercury volatilization after 10-16 days from Hagerstown silt loam amended to 5-19 ppm Hg as $HgCl_2$. The failure of reinoculation with microorganisms, or resupply with glucose or glucose plus KNO_3 to restimulate further Hg losses suggests that the stabilized Hg is no longer available for biological mobilization to a volatile form.

These soils show a high affinity for mercury. In excess of 97% of the Hg applied at 1 μg Hg/g soil was removed from aqueous solution by all of the soils in 24 hour equilibration studies. Thus, the adsorption of Hg(II) on a soil surface would not appear to prevent volatilization. The process by which the added Hg(II) is eventually stabilized may involve incorporation with the sulfhydryl groups of soil organic matter or precipitation as HgS.

The reason why the Campspass soils offers greater protection from volatile loss as compared to the Terry soil and others is not known. The high organic matter content of the Campspass (Table 21.2) is the most obvious factor, as organic matter tends to bind both Hg(II) and Hg^o strongly (Trost and Bisque, 1972; Gjessing, 1976). However, the nature of the microflora may also be a factor. It is interesting to note that the Terry soil shows greater resistance to inhibition of microbial respiration by Hg(II) amendment than the Campspass soil (see pages 818-820), and that microbial resistance to mercurials is often associated with ability of the organisms to detoxify their environment by volatilization of mercury (Vaituzis *et al.*, 1975). Mercury loss also appears to increase with increasing soil pH (Table 21.2). Frear and Dills (1967) showed the volatile loss of mercury from a series of limed Hagerstown silt loams amended with $HgCl_2$ to increase as soil pH increased from 5.3 to 6.4.

Soil Water Content and Soil Temperature as Factors in the Volatile Loss of Soil-Applied Inorganic Hg (II)

Volatilization of Hg was retarded at both very low and very high moisture contents (Figure 21.3). In the case of the drying soils, the loss process was reactivated to varying extents by rewetting the soils on day 25.

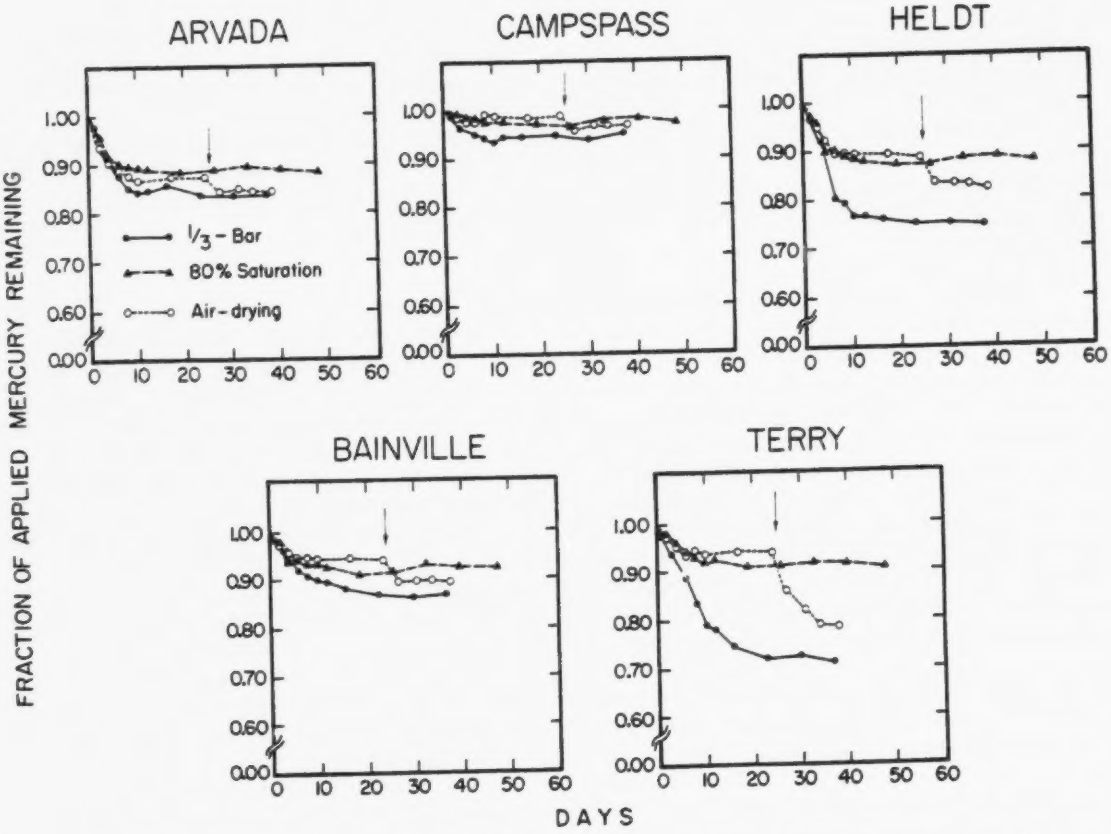


Figure 21.3. Effect of soil water content on Hg loss from mercuric nitrate-amended soils. Vertical arrow at day 25 indicates time of rewetting of air-dried soils.

As shielding of the γ-radiation by the water added at this time reduced the count rate by less than 1%, the rapid loss of Hg observed was judged not to be an artifact of the analytical method.

Figure 21.4 shows that at 35 C, the initial Hg loss rate was accelerated, but except in the case of the Heldt soil, the total loss was the same as that seen from samples equilibrated at the room temperature samples. At 10 C, the Hg loss rate and, in all but the Heldt soil, the total loss of Hg, was reduced with respect to the room temperature samples. Removal of these samples from the 10 C water bath after 5 1/2 weeks and subsequent storage at room temperature for 2 weeks appeared to stimulate further losses. The coefficient of variation in these experiments averaged 0.9%.

Mercury lost from the system is a composite of the Hg converted to a volatile form, minus that portion of the volatile Hg thus produced which is subsequently sorbed by the soil. Metallic mercury vapor (Hg^o) appears to be the predominant volatile species from Hg(II)-amended soils (Johnson and Braman, 1974; Rogers, 1975). The Bainville and Campspass soils have been shown (see Table 21.3) to sorb about twice as much Hg^o from air as the Heldt soil, and about four-times as much as the Arvada and Terry soils. Thus, the high losses of Hg observed in the Hg(II)-amended Terry soil may be the result of both a low degree of retention of Hg^o, as well as a high degree of conversion of Hg(II) to Hg^o. Similarly, the low losses of Hg seen here for the Campspass and Bainville soils are probably due, at least in part, to a high degree of soil retention of the Hg^o produced.

Earlier studies (see pages 802-805) which showed a stimulation of Hg-loss upon the addition of glucose to the soils, and a suppression of loss following autoclaving of the soils, suggest a major role for microorganisms in the volatile loss of applied Hg(II) from soils. A soil moisture content of 50 to 75% of field capacity is optimal for aerobic microbial activity (Alexander, 1962). The diminished Hg losses, with respect to those samples moistened to the 1/3-bar moisture content, seen in the air-dried and the 80% saturated soils (Figure 21.3) suggests that the activity of aerobic soil microorganisms is involved in the volatilization process, as a purely chemical reduction of Hg(II) to Hg^o would presumably be favored by high water contents. A water content - Hg volatilization relationship similar to that observed here was reported for the loss of Hg^o from a phenylmercuric acetate-amended sandy loam (Kimura and Miller, 1964).

Most soil microorganisms are mesophiles, with a temperature optimum of 25-37 C. Maximal respiratory activity in soils, as judged by the breakdown of carbonaceous materials, generally occurs in the 30-40 C range. At 10 C, below the growth range of most mesophiles, soil microbial activity will be considerably reduced (Alexander, 1962). Thus, the temperature-Hg loss relationships (Figure 21.4) also are consistent with a microbial action hypothesis, although vapor pressure and reaction kinetic considerations would also predict increasing loss rates with increasing temperature. The lower losses of Hg seen at 10 C may also reflect the higher degree of sorption of Hg^o (or other gaseous species) by the soils expected at lower temperatures.

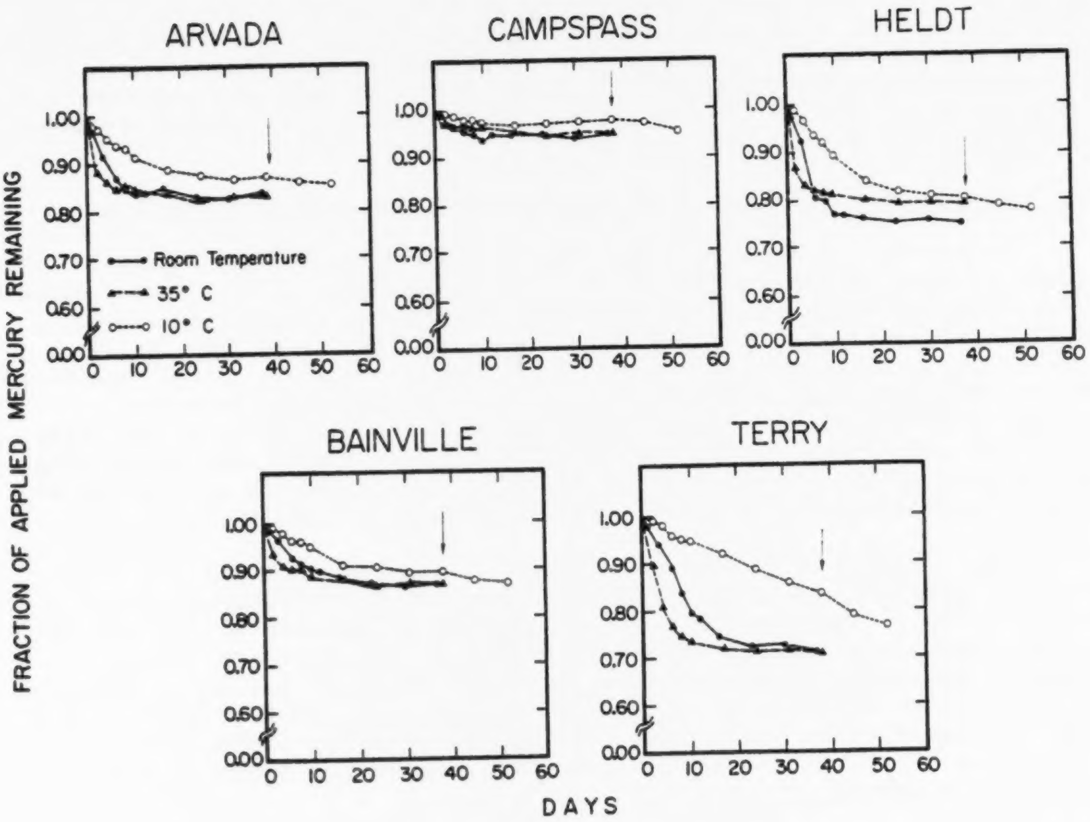


Figure 21.4. Effect of temperature on Hg loss from mercuric nitrate-amended soils. Vertical arrow at day 38 indicates time of initiation of room-temperature storage for samples previously equilibrated at 10 C.

The magnitude of mercury loss seen here is not unreasonable in light of other reports. Wimmer (1974) observed Hg losses of up to 40% in 12 weeks from soils amended to 0.2 ppm Hg by surface application of mercuric acetate. Gracey and Stewart (1974) and Hogg (1976) found 10-20% losses in 20-25 weeks in soils amended to 10 ppm Hg as $HgCl_2$. While several Hg loss studies have demonstrated first order kinetics over periods ranging from of 1 day to 27 months (Bothner and Carpenter, 1973; Alberts *et al.*, 1974), the rapid approach to equilibrium reported here and in earlier work (Frear and Dills, 1967) suggests the dubious validity of extrapolating short-term loss data on the assumption of first order behavior. For example, a semi-logarithmic plot of residual Hg vs. time for the first 10 days of data from the room temperature, 1/3-bar, Terry soil samples predicts an erroneous soil residence half-life of 30 days for the applied Hg.

The Volatile Loss of Mercury from Soils Amended with Methylmercury Chloride

Glucose and nitrate supplementation of the soils generally repressed volatile losses of mercury (Figure 21.5). Autoclaving of the soils supressed losses from the Arvada and Heldt soils but resulted in slightly increased losses from the Campspass and Bainville soils. Total losses from the autoclaved and non-autoclaved Terry soils were similar, although in the case of the non-autoclaved soil, most of the loss came in the period from 0 to 16 days after amendment, while in the case of the autoclaved soil, the major losses occurred in the period from 14 to 34 days after amendment, suggesting the recolonialization of the soil by microorganisms following autoclaving as a possible causative agent in the delayed volatile loss response.

Volatilization of mercury was retarded at both very low and very high moisture contents (Figure 21.6). Soils maintained at 80% saturation lost from 3 to 17% of their initial mercury amendment as compared to 7 to 44% for those maintained at the 1/3-bar moisture content. Air-drying of soils resulted in a termination of mercury loss after the first 6-8 days. Rewetting of the soils on day 28 to the 1/3-bar moisture content resulted in a renewed loss of mercury, with total losses over the two-month monitoring period approaching those of the soils maintained throughout this period at the 1/3-bar moisture content.

The magnitude of mercury loss increased with soil temperature over the 16 to 35 C range examined, with losses of up to 60% observed on the Terry soil at 35 C (Figure 21.7). In all cases (Figures 21.5, 21.6, 21.7), highest losses were observed in the strongly alkaline soils - Terry, Heldt and Arvada.

Work by Spangler *et al.* (1973 b) suggests that bacteria capable of degrading and volatilizing methylmercury (by reductive demethylation) under aerobic and/or anaerobic conditions are prevalent in the aquatic environment. Floyd and Sommers (1974) found that the addition of glucose, and to a lesser extent acetate, methanol or ethanol, to lake sediments increased the degradation of methylmercury chloride.

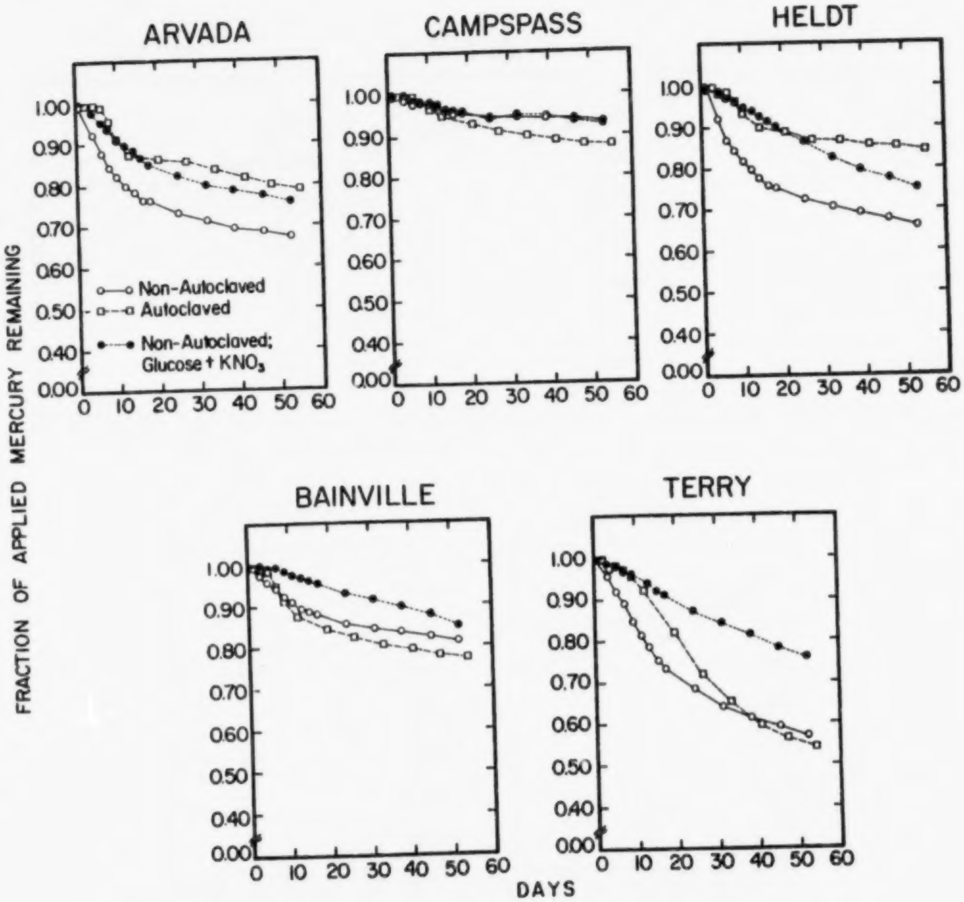


Figure 21.5. Effect of autoclaving and glucose + KNO_3 on Hg loss from soils amended with methylmercury chloride.

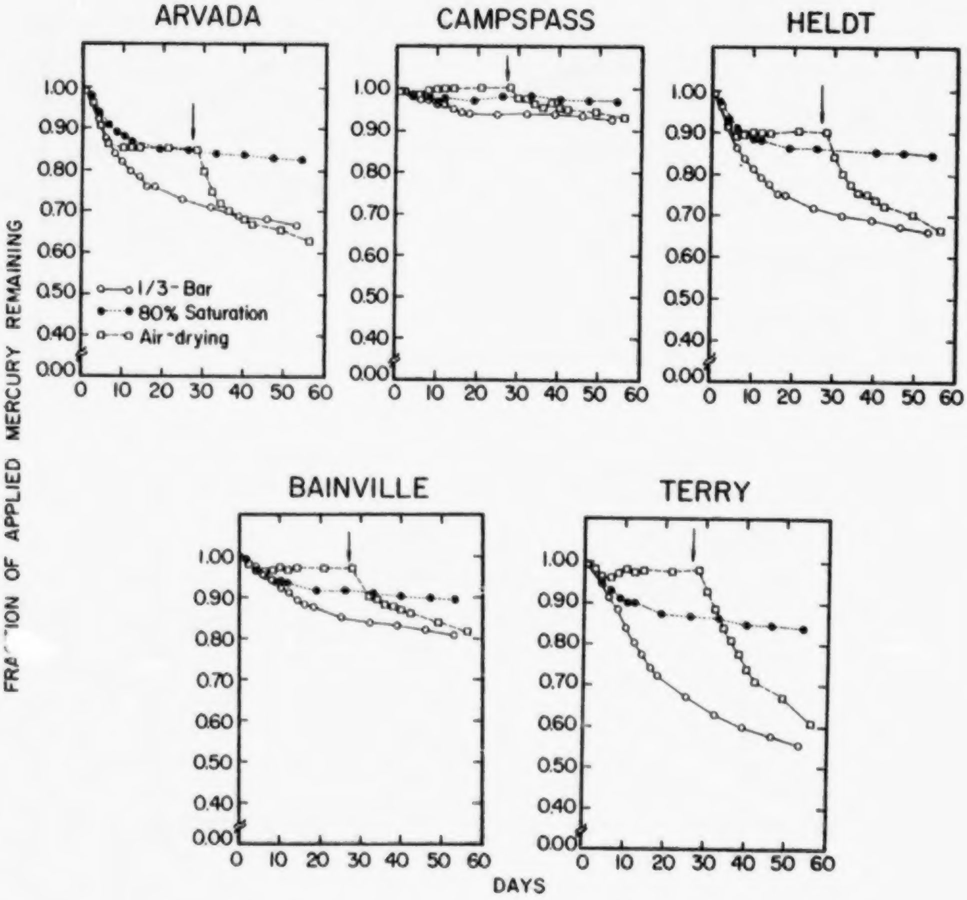


Figure 21.6. Effect of soil water content on Hg loss from soils amended with methylmercury chloride. Vertical arrow at day 28 indicates time of rewetting of air-dried soils.

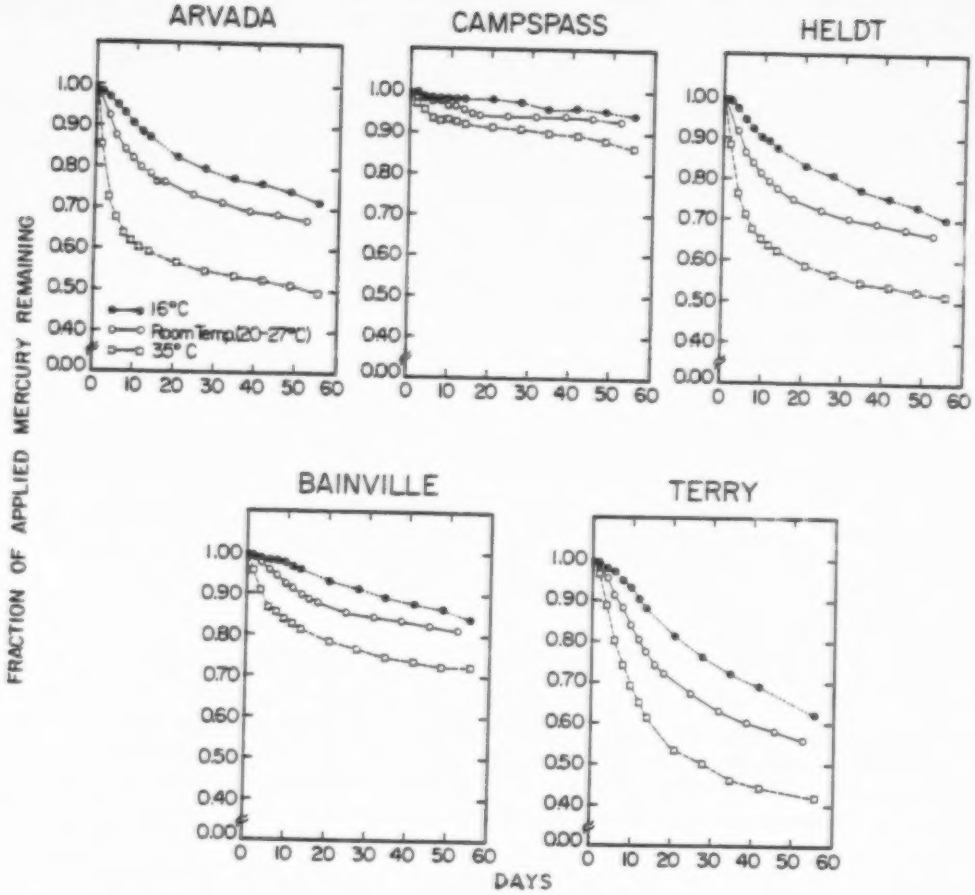


Figure 21.7. Effect of temperature on Hg loss from soils amended with methylmercury chloride.

In work reported earlier (see pages 802-805) we found glucose and glucose & nitrate supplementation to increase the volatilization of mercury from soils treated with mercuric nitrate. On the basis of the literature and our earlier experience with Hg^{2+}-amended soils, the finding here of suppressed mercury losses upon addition of glucose & nitrate to methylmercury-amended soils was surprising, as these metabolic substrates would be expected to stimulate the activity of microorganisms which could promote the volatile loss of mercury through either the reductive demethylation of methylmercury to methane and volatile metallic mercury, Hg^{o}, or the synthesis of volatile dimethylmercury.

The diminished losses observed here upon addition of glucose & nitrate may be the result of the stimulation of a biologically-mediated transformation of the methylmercury to a form (*e.g.* Hg^{2+}, or incorporated into microbial tissue) less subject to, but certainly not immune from, volatile loss processes. Billen *et al.* (1974) showed methylmercury to be stable in sterile river water, while after 90 hours in non-sterile water, only 10 to 20% of the initially-present Hg remained as methylmercury. Of the portion of the methylmercury which was transformed, from 50 to 80% was converted to dissolved (presumably Hg^{2+}) mineral mercury, with the remainder being lost from solution as volatile metallic mercury. Bache *et al.* (1973) found that at the end of a cropping experiment, 90% or more of the mercury present in mineral soils treated with methylmercury dicyandiamide was present as Hg^{2+} or some form other than intact methylmercury. This transformation was apparently not to Hg^{o} or $(CH_3)_2$ Hg, as no detectable volatile loss of mercury from the soils was observed. Experiments by Spanis *et al.* (1962) with autoclaved- and non-autoclaved soils showed the transformation of methylmercury dicyandiamide to a form of lesser fungicidal activity to be rapid (only 20% of the initial activity remaining after 4 days) and largely microbially-mediated.

As also seen with Hg^{2+}-amendment of these same soils (see pages   ), volatile losses of mercury following methylmercury-amendment were lower when soils were maintained at high moisture contents (80% saturation) or allowed to air-dry, as compared to those maintained at the 1/3-bar moisture content (Figure 21.6). The differences observed here between the air-dry and 1/3-bar conditions are in agreement with the observation by Rogers (1976) that methylmercury losses during the period 1 to 3 weeks after Hg^{2+}-amendment of a loam and clay soil increased as the soil moisture content increased from 25 to 75% of the moisture holding capacity.

Rowland *et al.* (1977) found that hydrogen sulfide stimulated the volatile loss from solution of methylmercury chloride, presumably as a sulfur-derivative of methylmercury. In soils at high moisture contents, H_2S might be produced in anaerobic microsites, thereby stimulating the volatile loss of mercury via such a mechanism. However the present data (Figure 21.6) do not support this view. Rogers (1976) showed that four times as much methylmercury was present in a water-saturated loam (amended with mercuric nitrate) as in the same soil at 75% of the moisture holding capacity. As the quantity of methylmercury present at any given time is the net result of production and loss, it is not clear if this observation is the result of greater methylmercury synthesis and/or less methylmercury loss at high moisture conditions.

Floyd and Sommers (1974) and Rogers (1976) found methylmercury loss from soils and sediments to increase with increasing temperature from 4 to 36 C. Figure 21.7 shows a similar response over the 15 to 35 C range studied here.

The pattern of mercury loss from these five soils following methylmercury-amendment shows some differences from that observed earlier with Hg^{2+}-amendment:
(1) Mercury loss from Hg^{2+}-amended soils generally ceased after about 2 weeks, while following methylmercury amendment, mercury loss was sustained for periods in excess of 7 weeks.
(2) While the same relative ordering of the soils with respect to volatile loss generally holds for both Hg^{2+}- and methylmercury-amendment (Terry > Heldt > Arvada > Bainville > Campspass), losses from the Arvada soil relative to the Terry and Heldt soils are much higher in the methylmercury case.
(3) As discussed earlier, glucose & nitrate supplementation of soils stimulated mercury loss from Hg^{2+}-amended soils, but suppressed it in methylmercury-amended soils.
(4) Volatile loss of mercury from methylmercury-amended soils showed a more pronounced response to higher temperature and the rewetting of air-dried soils than their Hg^{2+}-amended counterparts. At 35 C, as compared to room temperature, there was an increase in total mercury loss from methylmercury-treated soils, while with Hg^{2+}-treated soils, the initial rate of loss was increased, but the total quantity of mercury lost was unaffected, by increasing temperature.

These results indicate that methylmercury present in soils as a result of either direct contamination or in-situ synthesis may be subject to rapid and extensive losses to the atmosphere. Such environmental mobility should be recognized in any considerations of mercury cycling and persistence in soil systems.

The Retention of Metallic Mercury Vapor by Soils

Radioassay of the individual petri dishes of soil removed from the bell jar exposure system showed the coefficients of variation for the measured Hg uptakes of the 10 dishes of each soil type to be 4% or less, indicating that well-mixed conditions did indeed exist within the bell jar, and that the assumption of an ambient vapor concentration equal to that measured at the outlet is valid. The relative order of mercury sorption by the soils (Table 21.3) was:

Bainville>Campspass>Heldt>Arvada>Terry

The 2000 g of soil contained in the bell jar removed an average of 56% of the entering mercury vapor.

TABLE 21.3. UPTAKE OF MERCURY BY SOILS FOLLOWING 10 DAY EXPOSURE TO AIR STREAM CONTAINING 14.3 μg Hg^o/m^3 AIR[*]

SOIL	μg Hg/kg soil
Arvada	10.5 ± 1.7
Campspass	40.8 ± 1.0
Heldt	23.7 ± 2.4
Bainville	45.6 ± 2.7
Terry	9.5 ± 1.0

[*] Values shown are means of 3 replicates + 1 standard error.

No loss of the sorbed mercury from the soils maintained at air-dryness was observed over the 16 day monitoring period. Soils maintained at the 1/3-bar moisture tension showed maximal losses of about 5% of the total sorbed mercury. While such losses are quite small, they suggest that microbial activity stimulated by the addition of water to the soils may promote the biotransformation of the sorbed mercury to a more volatile species.

The majority of the sorbed mercury was liberated at temperatures between 100 and 200 C (Figure 21.8). Similar steep, S-shaped Hg-loss vs. temperature curves have been reported for several mercury compounds alone (Koksoy *et al.*, 1967), or mixed with soil (Koksoy and Bradshaw, 1969). Elemental mercury showed losses of 30-35% at 70-80 C, while mercurous and mercuric chloride showed losses of only 1-6% in this temperature range. All three compounds showed complete mercury volatilization below 250 C. In contrast, mercuric sulfide and oxide did not show any Hg-loss until the samples were heated to 210-270 C, with complete Hg-liberation at 340 and 535 C, respectively (Koksoy *et al.*, 1967). On the basis of this literature evidence, HgS and HgO appear to be unlikely candidates for the sorbed mercury species observed in the studies reported here.

Major volatile losses of Hg from the soils studied here occurred at a somewhat lower temperature range (100-200 C) than demonstrated by Koksoy and Bradshaw (1969) for soil samples taken from sites surrounding a cinnabar deposit in Turkey. Based on field evidence, these investigators postulated the gaseous dispersion of metallic mercury vapor away from the zone of mineralization, and its subsequent sorption by soil organic matter as an important mechanism for the Hg enrichment patterns observed in the soils of the secondary environment. These soils showed maximal Hg losses in the 200-300 C range.

Sodium hydroxide, hydrochloric acid and benzene removed the most sorbed mercury (Table 21.4). Little or no mercury was extractable by water, potassium

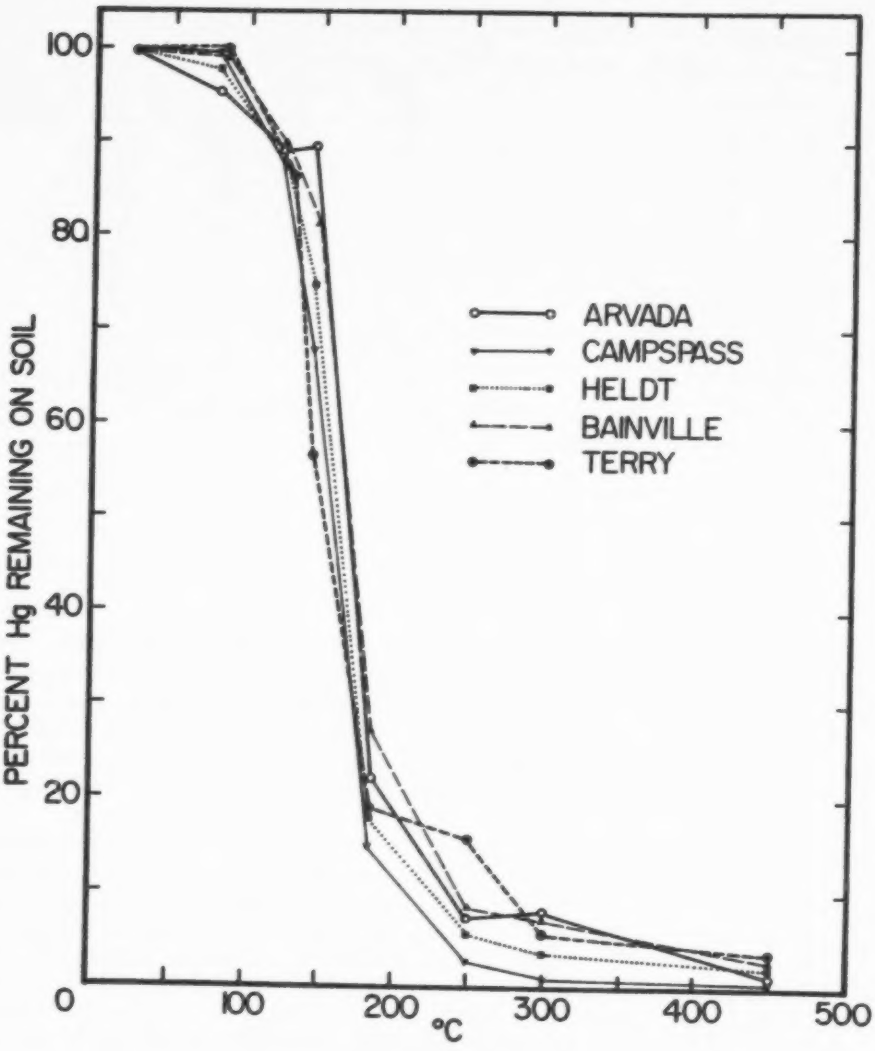


Figure 21.8. Volatile loss of sorbed mercury from soils as a function of temperature.

or calcium chloride, cupric acetate, methanol or DTPA, while cysteine, acetylacetone and cupferron showed limited extraction abilities.

TABLE 21.4. REMOVAL OF SORBED MERCURY VAPOR FROM SOILS BY VARIOUS CHEMICAL EXTRACTANTS*

		Hg Extracted				
Extractant	Soil	Arvada	Campspass	Heldt %	Bainville	Terry
(a) distilled water		0.1	0.2	0.4	0.3	0.4
(b) 1 *N* KCl		0.1	0.0	0.0	0.0	0.2
(c) 1 *N* $CaCl_2$		0.2	0.1	0.2	0.0	0.2
(d) 1 *N* HCl		71.1	15.8	1.2*	68.0	79.6
(e) 1 *N* NaOH		91.7	74.3	89.3	82.7	92.3
(f) 0.5 *M* acetylacetone		2.6	0.9	2.8	2.5	3.7
(g) 0.5 *M* cupferron		2.7	8.7	4.7	3.2	2.5
(h) 0.5 *N* Cu $(C_2H_3O_2)_2$		0.6	0.3	0.0	0.3	1.2
(i) DTPA:TEA:$CaCl_2$		0.2	0.4	0.5	0.5	0.1
(j) 0.1 *M* cysteine		4.0	3.7	5.3	3.1	2.9
(k) benzene		9.3	5.1	11.9	14.7	12.8
(l) methanol		1.1	0.4	0.3	0.1	0.7

*1.4 *N* HCl extracted 49.6%

*Values shown are means of 3 replicates.

While water soluble mercury compounds such as $HgCl_2$ have been identified in mineralized rock zones (Koksoy and Bradshaw, 1969), the failure of distilled water to extract the sorbed mercury (Table 21.4) makes such compounds unlikely candidates for the sorbed species observed here. The failure of KCl or $CaCl_2$ to remove the sorbed mercury indicates that no significant amount exists as a species which is readily available to ion exchange.

Hydrochloric acid extracted from 1 to 80% of the total sorbed mercury. The low Hg extraction from the Heldt soil is undoubtedly due to the reduction in the effective H^+ concentration resulting from reaction with the free lime present in this soil (Table 21.2). When the Heldt soil was extracted with 1.4*N* HCl, 50% of the sorbed mercury was released. Sodium hydroxide extracted 74 to 92% of the sorbed mercury from the soils. While both HCl and NaOH are rather non-selective extractants which can solubilize portions of both the mineral- and the organic- soil colloids, the uniformly high yield of sorbed mercury seen with the NaOH extractions suggests the association of the sorbed mercury species with the soil organic matter. Acidification of the NaOH extract (Stevenson, 1965) shows a large portion of the Hg thus removed from the soil to be associated (as a sorbed or coprecipitated species) with the insoluble, humic acid (Table 21.5). Whether the Hg present in the solution phase of the acidified NaOH extract exists as a species soluble in both acid and alkali, as a fulvic acid complex, or as a species which has been acid-leached from the humic acid is not clear. Only a limited portion (0.5-4%) of the Hg extractable by $1N$ HCl (or $1.4N$ HCl for the Heldt soil) partitioned into benzene upon shaking with the HCl extracts. Trost and Bisque (1972) have shown soil organic matter analogs, *e.g.* peat, humic acid, pine mull, etc. to have high Hg^0 sorption capacities.

TABLE 21.5. GROSS CHEMICAL FRACTIONATION OF SORBED Hg EXTRACTED FROM SOILS BY $1N$ NaOH

Soil	% of sorbed Hg in acidified NaOH extract associated with humic acid fraction
Arvada	46
Campspass	86
Heldt	58
Rainville	66
Terry	52

Due to their ability to chelate metals, aqueous solutions of acetylacetone and cupferron have proven to be good extractants of organic matter from podzolic B horizons (Martin and Reeve, 1957). Both of these reagents, in particular the cupferron with the Campspass soil, extracted a small portion of the sorbed mercury, lending further support to the existence of the mercury as an organo-mercury complex. However no sorbed Hg was extracted with cupric acetate, even though copper forms very stable complexes with organic matter (Stevenson and Ardakani, 1972).

The DTPA solution tested here has been used as a soil extractant for several trace elements including zinc, iron, manganese and copper (Follet and Lindsay, 1971). This chelate however was ineffectual as an extractant for the sorbed mercury. Cysteine, a monothiol amino acid, was selected for use because of the known affinity of mercury to form complexes with sulfhydryl groups. The 0.1 *M* cysteine solution extracted from 3-5% of the total sorbed mercury indicating that at least a portion of the sorbed mercury is available for complexation.

The selective extraction of organomercurials into organic solvents has long been used as the method for partitioning the total mercury content of biological materials into organic vs. inorganic mercury components (Miller *et al.*, 1958; Gage, 1961). The higher removals of sorbed mercury obtained with benzene as compared to methanol suggests the occurrence of at least a portion of the sorbed Hg as, or in association with, an organic compound with an affinity for non-polar as compared to polar organic solvents.

Work reported in section 22 (pages 856-861) showed the same relative order of Hg^o sorption for these same five soils. Using a technique developed by Clarkson and Greenwood (1970) for the selective determination of inorganic mercury in the presence of organomercurial compounds in urine, blood, and animal tissues, 20-27% of the sorbed mercury in the Arvada, Heldt and Terry soils, and 10% and 2% of the sorbed mercury in the Bainville and Campspass soils, respectively, was ascribed to an inorganic species. This evidence further supports the existence of a large portion of the sorbed mercury as an organo-complex.

The retention of Hg^o by soils as demonstrated here offers an explanation for the enrichment in Hg seen in the soils around a coal-fired power plant (Klein and Russell, 1973). In predictions of ground level air concentrations of mercury vapor used in evaluating power plant sitings, the effects of mercury vapor removal mechanisms have generally been neglected (Lyon, 1977). The data presented here indicate that the sorption of Hg^o by surface soils offers one such significant atmospheric removal mechanism. Such processes diminish the inhalation hazards associated with metallic mercury vapor to organisms downwind from the source. However, more information is needed regarding the abiotic and biotic cycling of this soil-sorbed mercury in order to fully assess its environmental impact.

The Effect of Mercuric Chloride on Carbon Mineralization in Soils

Table 21.6 shows the results of the short-term radiorespirometry trials. In all of the soils, amendments greater than 40 μg Hg/g soil were required to yield significant reductions in CO_2-evolution from glucose. The 200 μg Hg/g soil amendment reduced CO_2-production in the Arvada and Bainville soils, and 400 μg Hg/g soil was sufficient to depress glucose metabolism in all soils examined.

Several patterns of effect are seen in Table 21.7, showing the results of the month-long examination of CO_2-evolution from the $HgCl_2$ amended soils. The Terry soil shows essentially no inhibition of CO_2-production during the monitoring period. In the Arvada soil, inhibition of CO_2-production is evident during the first week at 1 ppm Hg and above; however, in succeeding weeks only the 100 ppm Hg amendment continues to show any depression. In contrast, the Campspass soil shows depressed CO_2-production at the 0.1 ppm Hg amendment and above for all but week 2. The Heldt soil shows no treatment effect during the first week, but marked inhibition at all levels of amendment for the succeeding 3 weeks; here the 1 ppm Hg treatment shows greater CO_2-production than the 0.1 ppm Hg treatment. The Bainville soil is unique in exhibiting increased CO_2 production with respect to the control at the 1 ppm Hg amendment during the first week; in succeeding weeks inhibition is seen only at progressively higher levels.

It is clear that exposure time and/or substrate type are important considerations in assessing the respiratory impact of mercuric chloride in these soils. All of the soils except the Terry showed suppressed CO_2-production throughout the month-long monitoring period at 100 ppm Hg, while only the Arvada and Bainville soils showed any such effect of 200 ppm Hg in the short-term exposure studies. Jeffries and Butler (1975) observed such

TABLE 21.6. SHORT-TERM EFFECTS OF MERCURIC CHLORIDE AMENDMENTS ON THE EVOLUTION OF CO_2 FROM GLUCOSE IN SOIL-WATER SUSPENSIONS

$HgCl_2$ concentration		ng CO_2/g soil/6 hours*				
M	µg Hg/g soil	Arvada	Campspass	Heldt	Bainville	Terry
0	0	18.9 a	18.0 a	9.9 a	19.2 ab	11.2 a
1×10^{-4}	40	16.3 a	15.2 a	9.7 a	21.2 a	12.8 a
5×10^{-4}	200	12.6 b	10.6 ab	6.2 ab	16.1 b	11.0 a
1×10^{-3}	400	4.8 c	5.9 b	3.9 b	8.4 c	3.5 b

*Means (4 replicates) in same column followed by same letter are not significantly different at the 5% level by Duncan's multiple range test.

TABLE 21.7. LONG-TERM EFFECTS OF MERCURIC CHLORIDE AMENDMENT ON CO_2 EVOLUTION FROM MOIST SOILS

Soil	µg Hg/g soil	mg CO_2/g soil/ week*			
	Week:	1	2	3	4
Arvada	0	0.65 a	0.41 a	0.47 a	0.31 a
	0.1	0.62 ab	0.35 a	0.33 a	0.25 ab
	1.0	0.56 bc	0.34 a	0.35 a	0.34 a
	10	0.52 c	0.32 a	0.32 a	0.25 ab
	100	0.43 d	0.26 b	0.22 b	0.17 b
Campspass	0	1.60 a	1.24 a	1.28 a	0.95 a
	0.1	1.45 b	1.22 a	1.13 b	0.85 b
	1.0	1.48 b	1.28 a	1.12 b	0.84 b
	10	1.40 b	1.24 a	1.00 c	0.80 b
	100	1.26 c	0.98 b	0.85 d	0.68 c
Heldt	0	0.33 a	0.47 a	0.33 a	0.45 a
	0.1	0.32 a	0.16 c	0.07 b	0.06 c
	1.0	0.34 a	0.28 b	0.30 a	0.18 b
	10	0.34 a	0.17 c	0.08 b	0.06 c
	100	0.36 a	0.18 c	0.10 b	0.06 c
Bainville	0	0.40 b	0.36 ab	0.43 ab	0.44 a
	0.1	0.36 c	0.39 a	0.48 a	0.42 a
	1.0	0.46 a	0.44 a	0.52 a	0.42 a
	10	0.36 c	0.30 bc	0.44 ab	0.31 a
	100	0.32 c	0.23 c	0.38 b	0.40 a
Terry	0	0.19 ab	0.21 a	0.16 a	0.16 a
	0.1	0.20 a	0.18 a	0.21 a	0.16 a
	1.0	0.22 a	0.22 a	0.19 a	0.20 a
	10	0.21 a	0.23 a	0.20 a	0.19 a
	100	0.18 b	0.18 a	0.16 a	0.17 a

* Means (3 replicates) in same column (for each soil) followed by same letter are not significantly different at the 5% level by Duncan's multiple range test.

an acute *vs.* chronic exposure dichotomy with the growth of the photosynthetic bacterium *Rhodopseudomonas capsulata*, where exposure in liquid medium for 20-80 minutes to levels of methylmercury acetate that were bacteriostatic for chronic (300 hour) exposures, stimulated growth.

The reasons for the differences in response of the soils studied are probably multifaceted. Differences in the binding ability of the soils will perhaps mitigate the toxic properties of the added mercury. As mercury tends to bind with the organic fraction of soils (D'Itri, 1972), the apparently greater sensitivity observed in the long-term study of the Campspass *vs.* the Terry soil, is surprising. However, the Campspass soil's much greater CO_2-production probably reflects a larger, and perhaps more varied microflora. As pointed out earlier, microoganisms vary widely in their sensitivity to mercurials. Strains of many environmentally-common procaryotic and eucaryotic genera have demonstrated mercury-tolerance, and the existence of such an array suggests resistance to mercurials may be common and widespread (Brunker, 1976). A possible genetic link exists between resistance to antibiotics and to mercury (Richmond and John, 1964). Zajic (1969) reports work which showed gram-negative bacteria to be more resistant than gram-positive bacteria to mercury penetration, suggesting that differences in the cell wall which influence the staining properties may also influence the uptake and toxicity of mercury. Thus, the nature of the indigenous microflora probably influences the tolerances observed in this study. Also the ability of the soils to lose by volatilization, presumably of Hg^{o}, mercury added as a mercuric salt by either biotic (Brunker and Bott, 1974) or abiotic (Alberts *et al.*, 1974) mechanisms, may influence the response observed to mercuric chloride amendment.

The Uptake from Soils of Inorganic Divalent Mercury by Plants

Plant/soil concentration factors ($CF = \frac{\mu g\ Hg/g\ tissue}{\mu g\ Hg/g\ soil}$) for Hg in the above ground portion of western wheatgrass seedlings range from 0.01 to 0.1 (Table 21.8). Tissue Hg concentrations were lowest for the Campspass and Arvada soils, the soils highest in organic matter and clay respectively, and highest in the high-lime Heldt soil. No toxicity symptoms or yield reductions with respect to control plants grown on unamended soils were evident. Tissue yields on the Arvada and Campspass soils were about double those on the Terry soil, with the Heldt and Bainville yields falling in the intermediate range.

The volatile loss of Hg, presumably as Hg^{o}, from soils amended with divalent mercury has been reported by several investigators (Rogers, 1975; Hogg, 1976). At the end of the 22 day growth period from 9 to 21 % of the initially-applied mercury had been lost from the soil-plant systems (Table 21.9). As the above-ground portion of the seedlings are exposed to Hg vapors emanating from the soil, the Hg found in the aerial tissue is probably the result of both foliar interception of volatilized Hg (see Section 22), as well as translocation from the roots.

TABLE 21.8. UPTAKE OF Hg BY ABOVE-GROUND PORTION OF WESTERN WHEATGRASS SEEDLINGS GROWN FOR 22-DAYS ON SOILS AMENDED WITH 1.0 µg Hg/g SOIL*

Soil Series	ng Hg/g DM
Arvada	31 ± 2.3
Campspass	12 ± 6.4
Heldt	96 ± 22
Bainville	40 ± 5.8
Terry	37 ± 8.3

*Tissue concentrations shown are means of 3 replicates ± standard error, and are calculated on a dry matter basis.

TABLE 21.9. PERCENT OF INITIALLY-ADDED Hg REMAINING IN SOIL*

Soil Series	% Hg remaining
Arvada	90.8 ± 0.4
Campspass	93.4 ± 0.2
Heldt	83.8 ± 0.2
Bainville	90.6 ± 0.5
Terry	79.4 ± 0.6

*At end of 22 day plant growth period.
Corrected for plant removal.
Values reported are means of 3 replicates ± standard error.

The concentration factors reported here are similar to those found for bromegrass (CF = 0.05-0.06) grown for 49 days on soils amended with 10 µg Hg (as $HgCl_2$)/g soil (Hogg, 1976), and for wheat and barley (CF = 0.04-0.11) grown to the heading-out or mature stage on soils amended with 0.5 µg Hg as $Hg(NO_3)_2$/g soil (Lee, 1974). Cadmium (Cd) is the Periodic Table neighbor of Hg in group IIb, and Munshower and Behan (1971) report a CF of 0.8 (based on acid extractable soil Cd) for western wheatgrass growing 20 miles from a zinc-cadmium smelter in Montana. Cd is generally considered to be more plant available than Hg (Lagerwerff, 1972).

In spite of the potential for both foliar and root uptake, only limited quantities of Hg were found in the above-ground portion of western wheatgrass grown on Hg-amended soils. Western wheatgrass would not appear to represent a source for food chain magnification of environmental mercury.

CONCLUSIONS

The experiments described here suggest that mercury added to the soil as a result of coal-combustion emissions may be subject to volatilization and reentry into the atmosphere. Such behavior may explain the mercury enrichment patterns seen in the soil surrounding the coal-fired power plant studied by Klein and Russell (1973). Soils have also been shown to scavenge metallic mercury vapor from the air. Thus the soil may act as both a source and a sink of mercury to and from the atmosphere.

Mercuric-mercury amendments of 0.1 ppm were shown to yield significant depression of microbial respiration in some of the soils examined. Based on an assumption of mercury deposition with the combustion particulate emissions, the Colstrip environmental analysis prepared by Westinghouse (1973) reports a maximal surface soil enrichment with respect to baseline soil mercury concentrations of less than 1%. While such enrichments seem trivial, most of the mercury probably does not deposit with the particulates and more importantly, the mercury emitted by the combustion of coal may be as a chemical form of greater toxicity and bioavailability than that present in the native soil. Future research efforts on trace element emissions from coal-fired power plants should be aimed at assessing the chemical form as well as total quantity of the various elements.

The work reported here on western wheatgrass and literature on other plant species suggests that root uptake of mercury is generally limited. However, the volatilization of mercury from the soil surface may increase the bioavailability of this mercury in the terrestrial environment, as Browne and Fang (Section 22) have shown the aerial tissues of plants to fix mercury vapor from the atmosphere.

REFERENCES

Abu-Erreish, G. M., E. I. Whitehead, and O. E. Olson. 1968. Evolution of Volatile Selenium from Soils. Soil Sci., 106:415-420.

Alberts, J. J., J. E. Schindler, R. W. Miller, and D. E. Nutter, Jr. 1974. Elemental Mercury Evolution Mediated by Humic Acid. Science, 184:895-897.

Alexander, M. 1962. Introduction to Soil Microbiology. John Wiley and Sons Inc., New York.

Bache, C. A., W. H. Gutenmann, L. E. St. John Jr., R. D. Sweet, H. H. Hatfield, and D. J. Lisk. 1973. Mercury and Methylmercury Content of Agricultural Crops Grown on Soils Treated with Various Mercury Compounds. J. Agr. Food Chem., 21:607-613.

Baker, K. F. 1962. Principles of Heat Treatment of Soil and Planting Materials. J. Aust. Inst. Agr. Sci., 28:118-126.

Ben-Bassat, D., and A. M. Mayer. 1975. Volatilization of Mercury by Algae. Physiol. Plant, 33:128-132.

Billen, G., C. Joiris, and R. Wollast. 1974. A Bacterial Methylmercury-Mineralizing Activity in River Sediments. Water Res., 8:219-225.

Billings, C. E., and W. R. Matson. 1972. Mercury Emissions from Coal Combustion. Science, 176:1232-1233.

Bothner, M. H., and R. Carpenter. 1973. The Rate of Mercury Loss from Contaminated Esturine Sediments in Bellingham Bay, Washington. Proc. First Annual NSF Trade Contaminants Conf. (Oak Ridge, Tenn.) pp. 198-210.

Brunker, R. L. 1976. Mercurial Toxicity in Yeast: Evidence for Catabolic Pathway Inhibition. Appl. Microbiol., 32:498-504.

Brunker, R. L., and T. L. Bott. 1974. Reduction of Mercury to the Elemental State by a Yeast. Appl. Microbiol., 27:870-873.

Clarkson, T. W., and M. R. Greenwood. 1970. Selective Determination of Inorganic Mercury in the Presence of Organomercurial Compounds in Biological Material. Anal. Biochem., 37:236-243.

Day, P. R. 1965. Particle Fractionation and Particle-Size Analysis. In: Methods of Soil Analysis, C. A. Black, ed. Agronomy, 9:545-567. Am. Soc. Agron., Madison, WI.

DeFilippis, L. F., and C. K. Pallaghy. 1975. A Simple Model for the Non-enzymatic Reduction and Alkyation of Mercuric Salts in Biological Systems. Bull. Environ. Contam. Toxicol., 14:32-37.

Diehl, R. C., E. A. Hattman, H. Schultz, and R. J. Haren. 1972. Fate of Trace Mercury in the Combustion of Coal. Bureau of Mines Tech. Progress Report (Managing Coal Wastes and Pollution Program) TPR-54. May, 1972. U.S. Dept. of the Interior.

D'Itri, F. M. 1972. The Environmental Mercury Problem. CRC Press, Cleveland.

Doran, J. W., and M. Alexander. 1977. Microbial Formation of Volatile Selenium Compounds in Soil. Soil Sci. Soc. Am. J., 41:70-73.

Eshleman, A., S. M. Siegel, and B. Z. Siegel. 1971. Is Mercury from Hawaiian Volcanoes a Natural Source of Pollution? Nature, 233:471-472.

Floyd, M., and L. E. Sommers. 1974. Mercury Transformation in Lake Sediments. Agron. Abstracts, p. 128. Am. Soc. of Agron., Madison, WI.

Follett, R. H., and W. L. Lindsay. 1971. Changes in DTPA-extractable Zinc, Iron, Manganese and Copper in Soils Following Fertilization. Soil Sci. Soc. Am. Proc., 35:600-602.

Frear, D. E. H., and L. E. Dills. 1967. Mechanism of the Insecticidal Action of Mercury and Mercury Salts. J. Econ. Entomol., 60:970-974.

Gage, J. C. 1961. The Trace Determination of Phenyl- and Methylmercury Salts in Biological Material. Analyst, 86:457-459.

Gjessing, E. T. 1976. Physical and Chemical Characteristics of Aquatic Humus. Ann Arbor Science Publishers Inc., Ann Arbor, Mich.

Gracey, H. I., and J. W. B. Stewart. 1974. The Fate of Applied Mercury in Soil. Proc. Int. Conf. Land Waste Management (Ottawa, Canada), pp. 97-103.

Hardcastle, J. E., and N. Mavichakana. 1974. Uptake of Mercuric Chloride and Methylmercury Chloride from Liquid Media by *Aspergillus niger* and *Penicillum notatum*. Bull. Environ. Contam. Toxicol., 11:456-460.

Hogg, T. J. 1976. The Fate of Applied Mercury in the Soil-Plant System Under Sewage Effluent Irrigation Conditions. M.Sc. Thesis. Univ. of Saskatchewan, Saskatoon.

Hughes, M. N. 1972. The Inorganic Chemistry of Biological Processes, John Wiley & Sons, London.

Jacobs, L. W., and D. R. Keeney. 1974. Methylmercury Formation in Mercury-treated River Sediments During in situ Equilibration. J. Environ. Qual., 3:212-226.

Jeffries, T. W., and R. G. Butler. 1975. Growth Inhibition of *Rhodopseudomonas capsulata* by Methylmercury Acetate. Appl. Microbiol., 30:156-158.

Jepsen, A. F. 1973. Measurements of Mercury Vapor in the Atmosphere. In: Trace Elements in the Environment, E. L. Kothny, ed. Adv. in Chem. Series No. 123. Am. Chem. Soc., Washington, D.C.

Johnson, D. L., and R. S. Braman. 1974. Distribution of Atmospheric Mercury Species Near Ground. Environ. Sci. Technol., 8:1003-1009.

Kauffman, M. D., and E. H. Gardner. 1976. Methods of Soil Analysis Used in the Soil Testing Laboratory at Oregon State University. Spec. Rep. 321, Oregon Ag. Expt. Sta., Corvallis.

Kimura, Y., and V. L. Miller. 1964. The Degradation of Organomercury Fungicides in Soil. J. Agr. Food Chem., 12:253-257.

Klein, D. H., and P. Russell. 1973. Heavy Metals: Fallout Around a Power Plant. Environ. Sci. Technol., 7:357-358.

Kokke, R. 1974. Micropollutant Interaction with Microbes, pp. 81-85. In: Isotope Tracer Studies of Chemical Residues in Food and the Agricultural Environment, I.A.E.A., Vienna.

Koksoy, M., and P. M. D. Bradshaw. 1969. Secondary Dispersion of Mercury from Cinnabar and Stibnite Deposits, West Turkey. Colorado School Mines Quart., 64:333-336.

Koksoy, M., P. M. D. Bradshaw, and J. S. Tooms. 1967. Notes on the Determination of Mercury in Geological Samples. Inst. Mining Metall. Trans/Sect. B (Bull. No. 726), 76:B121-124.

Lagerwerff, J. V. 1972. Lead, Mercury and Cadmium as Environmental Contaminants. pp. 593-636. In: Micronutrients in Agriculture, J. J. Mortvedt, P. M. Giordano and W. L. Lindsay (eds.). Soil Sci. Soc. Amer. Madison, WI.

Landa, E.R. and S.C. Fang. 1978. Effect of Mercuric Chloride on Carbon Mineralization in Soils. Plant and Soil, 49: 179-183.

Lee, C. C. 1974. ^{203}Hg Tracer Studies on Mercury Uptake from Soil by Wheat and Barley. Bull. Environ. Contam. Toxicol., 11:551-553.

Lyon , W. S. 1977. Trace Element Measurements at the Coal-fired Steam Plant. CRC Press Inc., Cleveland.

Magos, L. 1966. Radiochemical Determination of Metallic Mercury Vapour in Air. Brit. J. Ind. Med., 23:230-236.

Martin, A. E., and R. Reeve. 1957. Chemical Studies on Podzolic Illuvial Horizons. (I) The Extraction of Organic Matter by Organic Chelating Agents. J. Soil Sci., 8:268-278.

Martin, N. L. 1969. Western Wheatgrass. Range Plant Leaflet 76, Cooperative Extension Service, Oregon State University, Corvallis.

McCarthy, J. H. Jr., J. L. Meuschke, W. H. Ficklin, and R. E. Learned. 1970. Mercury in the Atmosphere. In: Mercury in the Environment. U.S. Geological Survey Professional Paper 713.

Miller, V.L., D. Lillis, and E. Csonka. 1958. Microestimation of Intact Phenylmercury Compounds in Animal Tissue. Anal. Chem., 30: 1705-1706.

Montana State Department of Natural Resources and Conservation. 1974. Draft Environmental Impact Statement on Colstrip Electric Generating Units 3 and 4. Vol. 3A. pp. 215-233.

Munshower, F. F., and M. J. Behan. 1971. Ecological Compartmentation of Airborne Cadmium in a Grassland Ecosystem (abstr.). Am. J. Bot., 58:476.

Olson, B. H., and R. C. Cooper. 1976. Comparison of Aerobic and Anaerobic Methylation of Mercuric Chloride by San Francisco Bay Sediments. Water Res., 10:113-116.

Piperno, E. 1975. Trace Element Emissions: Aspects of Environmental Toxicology. In: Trace elements in Fuel, S. P. Babu, ed. Advances in Chemistry Series - no. 141. Amer. Chem. Soc., Washington, D.C.

Peterson, G. H. 1962. Respiration of Soil Sterilized by Ionizing Radiations. Soil Sci., 94:71-74.

Richmond, M. H., and M. John. 1964. Co-transduction by a Staphylococcal Phage of the Genes Responsible for Penicillinase Synthesis and Resistance to Mercury Salts. Nature, 202:1360-1361.

Rissanen, K. 1973. Transformations of Inorganic Mercury in Soil. Report to I.A.E.A., Nov. 1973 (as cited by T. J. Hogg. 1976. The Fate of Applied Mercury in the Soil-plant System Under Sewage Effluent Irrigation Conditions. M.Sc. Thesis. Univ. of Saskatchewan, Saskatoon).

Robertson, D. E., E. A. Crecelius, J. S. Fruchter, and J. D. Ludwick. 1977. Mercury Emissions from Geothermal Power Plants. Science, 196:1094-1097.

Rogers, R. D. 1975. Methylation of Mercury in a Terrestrial Environment. Abstr. Int. Conf. on Heavy Metals in the Environment (Toronto, Canada) C-218.

Rogers, R. D. 1976. Methylation of Mercury in Agricultural Soils. J. Environ. Qual., 5:454-458.

Rowland, I. R., M. J. Davies, and P. Grasso. 1977. Volatilization of Methylmercuric Chloride by Hydrogen Sulphide. Nature, 265:718-719.

Sayler, G. S., J. D. Nelson, Jr., and R. R. Colwell. 1975. Role of Bacteria in Bioaccumulation of Mercury in the Oyster *Crassostrea virginica*. Appl. Microbiol., 30:91-96.

Schroeder, H. A. 1970. Cadmium, Zinc and Mercury. Air quality monograph 70-16. Amer. Petroleum Institute. Washington, D.C.

Siegel, S. M., and B. Z. Siegel. 1975. Geothermal Hazards. Mercury Emission. Environ. Sci. Technol., 9:473-474.

Slatyer, R. O. 1971. Effect of Errors in Measuring Leaf Temperature and Ambient Gas Concentration on Calculated Resistance to CO_2 and Water Vapor Exchange in Plant Leaves. Plant Physiol., 47:269-274.

Spangler, W. J., J. L. Spigarelli, J. M. Rose, and H. M. Miller. 1973a. Methylmercury: Bacterial Degradation in Lake Sediments. Science 180:192-193.

Spangler, W. J., J. L. Spigarelli, J. M. Rose, R. S. Flippin, and H. H. Miller. 1973b. Degradation of Methylmercury by Bacteria Isolated from Environmental Samples. Appl. Microbiol., 25:488-493.

Spanis, W. C., D. E. Munnecke, and R. A. Solberg. 1962. Biological Breakdown of Two Organic Mercurial Fungicides. Phytopathology, 52:455-462.

Stevenson, F. J. 1965. Gross Chemical Fractionation of Organic Matter. In: Methods of Soil Analysis, C. A. Black, ed. Agronomy, 9:1409-1421. Am. Soc. of Agron., Madison, Wis.

Stevenson, F. J., and M. S. Ardakani. 1972. Organic Matter Reactions Involving Micronutrients in Soils. pp. 79-114. In: Micronutrients in Agriculture, J. J. Mortvedt, P. M. Giordano and W. L. Lindsay, eds. Soil Sci. Soc. Amer., Madison, Wis.

Stozky, G. 1965. Microbial Respiration. In: Methods of Soil Analysis, C. A. Black, ed. Agronomy, 9:1550-1572. Am. Soc. Agron., Madison, WI.

Swanson, V. E., C. Huffman, Jr., and J. C. Hamilton. 1974. Composition and Trace Element Content of Coal, Northern Great Plains Area. U.S. Dept. Interior open file report, Northern Great Plains Resource Program, Mineral Resources Work Group Report (Feb. 1974) pp. 52-83.

Thayer, S. S. 1976. Volatilization of Mercuric Chloride by *Euglena gracilis A.* Abstr. Am. Soc. Pl. Physiol., Western Section Meeting (Missoula, MT) p. 6.

Tonomura, K., K. Maeda, F. Futai, T. Nakagami, and M. Yamada. 1968. Stimulative Vaporization of Phenyl-mercuric Acetate by Mercury-resistant Bacteria. Nature, 217:644-646.

Trost, P. B., and R. E. Bisque. 1972. Distribution of Mercury in Residual Soils. pp. 178-196. In: Environmental Mercury Contamination, R. Hartung and B. D. Dinman, eds. Ann Arbor Science Publisher Inc., Ann Arbor, Mich.

U. S. Dept. of Interior. 1973. Final Environmental Statement for the Geothermal Leasing Program, v. 1, chapt. III, pp. 16-22.

U.S. Salinity Laboratory Staff. 1954. Methods for Soil Characterization. pp. 83-126. In: Diagnosis and Improvement of Saline and Alkali Soils, L. A. Richards, ed. Agric. Handbook No. 60, USDA. U.S. Government Printing Office, Washington, D.C.

Vaituzis, A., J. D. Nelson, Jr., L. W. Wan, and R. R. Colwell. 1975. Effects of Mercuric Chloride on Growth and Morphology of Selected Strains of Mercury-resistant Bacteria. Appl. Microbiol., 29:275-286.

Van Faassen, H. G. 1973. Effects of Mercury Compounds on Soil Microbes. Plant Soil, 38:485-487.

Van Faassen, H. G. 1975. Methylation of Mercury Compounds in Soil, Sediment and Sewage Sludge Samples. Plant Soil, 44:505-509.

Westinghouse Environmental Systems. 1973. Colstrip Generation and Transmission Project. Applicant's environmental analysis.

Wimmer, J. 1974. Movement and Leaching of Mercury in Soil. Die Bodenkultur 25:369-379 (Ger.).

Zajic, J. E. 1969. Microbial Biogeochemistry. Academic Press, New York.

Zimmerman, P. W., and W. Crocker. 1933. The Injurious Effect of Mercury Vapor from Bichloride of Mercury in Soil or Rose Houses. Boyce Thompson Inst. Profess. Papers 1 (no. 23): 222-225. Yonkers, NY.

Zimmerman, P. W., and W. Crocker. 1934. Plant Injury Caused by Vapors of Mercury and Compounds of Mercury. Contrib. Boyce Thompson Inst. 6:167-187. Yonkers, NY.

SECTION 22

UPTAKE OF METALLIC MERCURY VAPOR BY SOILS AND VARIOUS PLANT SPECIES

C. L. Browne and S. C. Fang

ABSTRACT

Laboratory experiments were conducted to study (1) the sorption of metallic mercury vapor by dry soils, minerals and organic materials in relation to mercury vapor concentration or duration of exposure; (2) the transformation of the sorbed mercury and availability for plant uptake; and (3) the foliage uptake of mercury vapor by various plant species as affected by a number of environmental factors.

Both organic matter and mineralogical make-up of the soil appeared to play an important role in the wide range of mercury vapor sorption observed for the dry soils. The sorption phenomenon was adequately described by the Freundlich equation. Only a small fraction of mercury sorbed was transformed to mercuric mercury, the form most available for root uptake.

Mercury vapor uptake from the atmosphere by plants was found to be almost exclusively confined to leaves and, as such, a simple gaseous exchange model was employed to describe the phenomenon. The rate of mercury vapor uptake was dependent on ambient mercury vapor concentration, illumination level, and leaf temperature. Illumination level influenced leaf resistance to mercury vapor entry, but except in darkness, the effect on rate of uptake was small compared to that of leaf temperature, which affected internal biochemical and physical processes involved in the conversion of the metal to mercuric mercury. *In vitro* studies with homogenized leaf tissue demonstrated that this conversion was to a large extent enzymatically controlled. Uptake of mercury vapor was found to differ between plant species, but the most pronounced difference was that which existed between plants possessing C_3 and C_4 photosynthetic pathways.

INTRODUCTION

Because of abundant resources in the United States at relatively low cost, coal is being used as the primary fossil fuel for the production of energy. Although the mean mercury content in coal is small, more than 90% of mercury escapes into the atmosphere as vapors after burning (Mercury and the Environment). As mercury pollution of the entire ecosystem continues to increase through natural and man-made sources, a basic understanding of the cycling of mercury in the air-soil-vegetation system is urgently needed to complete the environmental picture. This report describes laboratory experiments designed to study the effect of physical and biochemical factors on the removal of mercury vapor from the atmosphere by plants and soils. Ambient temperature, ambient mercury concentration, illumination, and duration of exposure were studied in relation to their effect on components of a proposed model for gaseous mercury uptake by whole plants. At the cellular level, homogenized leaf samples were employed in an *in vitro* study of possible biochemical processes involved in the uptake of mercury vapor. Soils of differing characteristics, clay minerals, and various organic materials were also examined with respect to their sorption capacity for mercury vapor.

Theory of Mercury Vapor Uptake by Plants

Gas Assimilation Model

The entry of mercury vapor into plants can be expected to follow the same major transfer pathways as water vapor and carbon dioxide. As such, the model employed to describe mercury vapor uptake in this work was of similar form to one commonly used in carbon dioxide assimilation studies (Bierhuizen and Slatyer, 1964) in that,

$$U(Hg) = \frac{C_{A.Hg} - C_{L.Hg}}{r_{L.Hg} + r_{M.Hg}} \qquad (22.1)$$

where U(Hg) is the rate of mercury uptake per unit leaf surface (g cm^{-2} sec^{-1}); $C_{A.Hg}$ is the ambient mercury vapor concentration (g cm^{-3}); $C_{L.Hg}$ is the mercury concentration at immobilization sites within the plant and is assumed to be zero; $r_{L.Hg}$ is the total leaf resistance to mercury vapor exchange (sec cm^{-1}) and includes several component resistances, the principal of which are the stomatal, cuticular, and external boundary layer resistances; $r_{M.Hg}$ (sec cm^{-1}) is a residual term to account for unexplained physical and biochemical resistances to mercury vapor uptake.

A principal step in establishing the model was the determination of $r_{L.Hg}$, which was assumed to be related to total leaf resistance to water vapor exchange ($r_{L.H_2O}$) by the relationship,

$$r_{L.Hg} = r_{L.H_2O} \times \frac{D^o_{H_2O}}{D^o_{Hg}} \quad (\text{sec cm}^{-1}) \qquad (22.2)$$

where $D^o_{H_2O}$ and D^o_{Hg} are the free diffusion coefficients for the respective vapors (Jarvis, 1971). A value for D^{o}_{Hg} at 25 C of 0.15 cm^2 sec^{-1} was obtained from the results of Mikhailov and Kochegarova (1967), where experimental values of D^{o} were expressed as a function of temperature. This gives the ratio $D^{o}_{H_2O}/D^{o}_{Hg}$ a value of 1.73, where $D^{o}_{H_2O}$ at 25 C is 0.26 cm^2 sec^{-1} (Van Haveren and Brown, 1972). This ratio was effectively constant ($\pm$1%) over the range of ambient temperatures examined in our experiments. As Mikhailov and Kochegarova (1967) observed, there is a substantial difference between values of D^{o}_{Hg} derived experimentally and those which would be expected on a theoretical basis. Their data, however, corresponds with other published values for D^{o}_{Hg} (Jost, 1952; Spier, 1940).

In using equation 22.2, it was assumed that the diffusion coefficients were independent of stomatal pore size, and that the boundary layer resistance comprised but a small part of total leaf resistance. This second assumption is reasonably valid in a well ventilated chamber, such as was used in these experiments. The first assumption may not be particularly valid (Cowan and Milthorpe, 1968), and could likely lead to the greatest errors in estimation of $r_{L.Hg}$ in instances of narrowest stomatal opening. Such an assumption was expedient, however, in view of the complex and arbitrary alternative of calculating and employing diffusion coefficients appropriate to changing stomatal dimensions (Cowan and Milthorpe, 1968; Milthorpe and Penman, 1967).

Whole Plant Resistance to Gaseous Exchange

Since in this study it was desired to examine mercury uptake and subsequent distribution on whole-plant basis, it was necessary to determine leaf resistance to mercury vapor exchange ($r_{L.Hg}$), which in turn required the determination of total leaf resistance to water vapor exchange ($r_{L.H_2O}$, equation 22.2). Estimation of $r_{L.H_2O}$ is often confined to single-leaf chambers, one reason being the theoretical necessity to measure leaf temperature, a difficult entity to characterize accurately. An alternative theory was therefore sought whereby $r_{L.H_2O}$ of whole plants could be estimated in a plant chamber independently of leaf temperature measurement. Such a theory was proposed by Jarvis (1971). The approach involves determination of components of the basic transfer equation,

$$q_v = \frac{C_L - C_A}{r_{L.H_2O}} \tag{22.3}$$

where q_v is the flux of water vapor per unit of leaf surface (g cm^{-2} sec^{-1}), C_A is the ambient water vapor concentration (g cm^{-3}), and C_L is the water vapor concentration (g cm^{-3}) at evaporative surfaces within the leaf and is considered to be saturated at leaf temperature.

Jarvis' proposal is that by inducing a small change in ambient water vapor concentration, then

$$q_v' = \frac{C_L' - C_A'}{r_{L.H_2O}} \tag{22.4}$$

The assumption is that $r_{L.H_2O}$ is independent of change in ambient humidity. By subtracting equations 22.3 and 22.4,

$$r_{L.H_2O} = \frac{C_A' - C_A}{q_v - q_v'} - \frac{C_L' - C_L}{q_v - q_v'} \tag{22.5}$$

or

$$r_{L.H_2O} = \frac{\Delta C_A}{\Delta q_v} - \frac{\Delta C_L}{\Delta q_v} \tag{22.6}$$

Assuming that the change in leaf temperature is negligible, then the second term in the above equations can also be considered to be negligible such that

$$r_{L.H_2O} \approx \frac{\Delta C_A}{\Delta q_v} \tag{22.7}$$

This assumption concerning leaf temperature becomes more valid when dimensions of the leaves are small and ventilation is high (Gates, 1968). Use of equation 22.7, however, would result in some overestimation of $r_{L.H_2O}$ if $\Delta C_L/\Delta q_v$ had a finite value. No attempt has been made in these studies to quantify the value of this term by measurement of change in leaf temperature. The inherent error has been reduced, however, by the use of small plants and rapid stirring of air within the chamber. The principal assumption that $r_{L.H_2O}$ is a constant would also result in error if incorrect. This possibility is further discussed in the light of results of preliminary experiments in which the efficacy and versatility of the approach were examined.

MATERIALS AND METHODS

Plant Chamber

A schematic diagram of the plant chamber and associated equipment is presented in Figure 22.1. The cylindrical glass chamber was 12 cm in diameter and 30 cm in height (3.4 liters). The internal surfaces of the chamber and ancillary glassware were smeared very lightly with petroleum jelly to minimize adsorption of both water and mercury vapor. Air entering the open system was drawn from laboratory supply lines, dried, and then controllably mixed with water vapor saturated at chamber temperature to attain a desired humidity. Prior to entry of air into the chamber, by-pass systems were used to generate radioactive mercury vapor (^{203}Hg) as well as determine mercury and water vapor concentrations. Air within the chamber was rapidly mixed by a fan 7 cm in diameter (c. 800 rpm) so that the outlet vapor concentrations of water and mercury were assumed to be the ambient concentrations (Slatyer, 1971). Conditions were sufficiently turbulent so as to produce visible agitation of the leaves. Air flow rates were controlled by regulated flowmeters which were of the float-type and were regularly cleaned. Chamber temperature was controlled (± 0.02 C) by means of a water jacket serviced by a temperature-regulated water bath and circulator (Polyscience Corporation).

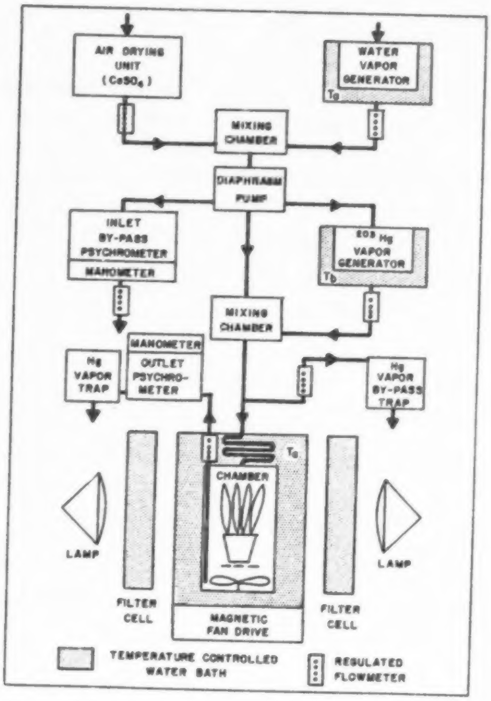

AIR DRYING UNIT ($CaSO_4$)
WATER VAPOR GENERATOR
T_a
MIXING CHAMBER
DIAPHRAGM PUMP
INLET BY-PASS PSYCHROMETER
MANOMETER
203 Hg VAPOR GENERATOR
T_b
MIXING CHAMBER
MANOMETER
OUTLET PSYCHRO-METER
Hg VAPOR TRAP
Hg VAPOR BY-PASS TRAP
T_a
CHAMBER
LAMP
LAMP
FILTER CELL
FILTER CELL
MAGNETIC FAN DRIVE
TEMPERATURE CONTROLLED WATER BATH
REGULATED FLOWMETER

Figure 22.1. Schematic diagram of the plant chamber for estimation of whole-plant resistances to water and mercury vapor exchange. The plant chamber and water vapor generator were serviced by the same water bath. The outlet psychrometer, although indicated separately, was incorporated within the plant chamber.

Illumination was provided by two banks of one or two reflector-type 150 watt incandescent lamps (G.E. Plant Light). These were laterally located, and the light was filtered through flowing water cells 7 cm in width. Illumination level was measured with a CdS photocell and was controlled by the number of lamps together with gauze screens.

Psychrometry

Vapor pressure of air entering and exiting the plant chamber was determined from components of the psychrometric equation,

$$e = e_w^o\ (T_w) - PA_w\ (T-T_w) \qquad (22.8)$$

where

e = vapor pressure (mm Hg)
$e_w^o(T_w)$ = saturated vapor pressure corresponding to T_w (mm Hg)
T_w = wet bulb temperature (C)

T = ambient temperature (C)
P = barometric pressure (mm Hg)
A_w = psychrometric constant (C^{-1})

Psychrometry was simplified by the large chamber size and a high outlet flow rate. This permitted use within the chamber of wet and dry bulb thermometers with a limit of reading by estimation of 0.05 C. Thermometers were ventilated and protected from direct radiation by cylindrical foam and aluminum foil shields; the wet bulb thermometer being located in the outlet and of a design so as to create high turbulent flow over the wick in the axial direction. The inlet by-pass psychrometer was of the same design and all thermometers were matched and calibrated over the range of operative temperatures.

Air flow through the inlet psychrometer was maintained at a velocity of 3.3 m sec^{-1} so that the psychrometric constant (A_w inlet) was assumed to be 6.6×10^{-4} $(1 + 0.00115\ T_w)$ C^{-1} (Tanner, 1972). A psychrometric constant (A_w outlet) was generated for the plant chamber by calibration against A_w inlet for every change in wicks. The relationship between these constants was unaffected by temperature and illumination, but particularly influenced by flow rate exiting the chamber (Figure 22.2). An exit flow of 1500 ml min^{-1} was therefore maintained in all experiments, this being satisfactory for adequate wet bulb depression as well as for trapping of mercury vapor.

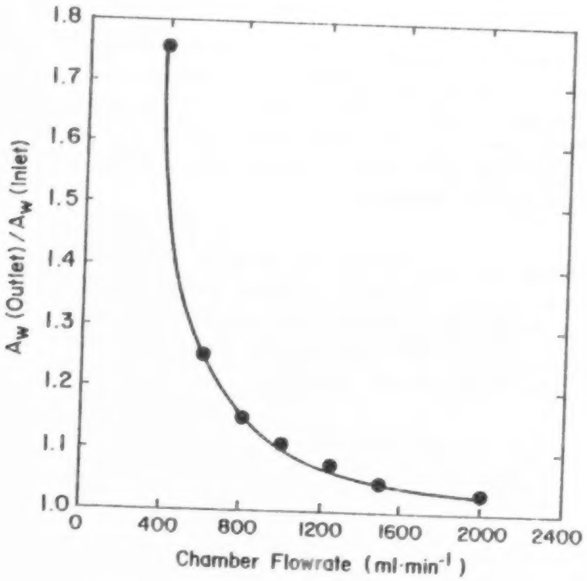


Figure 22.2. Effect of chamber air flow rate on the relationship between inlet and outlet psychrometric constants (A_w). The value of A_w (inlet) was 6.6×10^{-4} $(1 + 0.00115\ T_w)$ C^{-1}.

Inlet and outlet vapor pressures were converted to units of absolute humidity (ρ) using the relationship

$$\rho = 2.89 \times 10^{-4} \, [e/T] \; (\mathrm{g\ cm^{-3}}) \tag{22.9}$$

where T was the Kelvin temperature of the chamber (Slatyer, 1967).

^{203}Hg Vapor Generation

Prior to entry into the plant chamber, part (200 ml min^{-1}) of the total flow (1700 ml min^{-1}) was passed over a ^{203}Hg labelled mercury source. The resulting vapor was remixed with the bulk flow and a further 200 ml min^{-1} then diverted to determine the precise concentration of the mercury vapor which entered the chamber at a flow rate of 1500 ml min^{-1}. The temperature of the mercury source, and hence vapor concentration, was controlled by a thermoregulated water bath. To maintain a constant vapor concentration, it was also necessary to continuously and gently vibrate the source. Under this system, a given mercury vapor concentration could be reproduced to within ± 13%. Two sources were used during the course of the experiments, the first (170 mg) having an initial specific activity of 0.17 mCi/mg, and the second (33 mg) an initial specific activity of 0.06 mCi/mg.

Mercury vapor exiting the open system, which was located within a ventilated hood, was collected in traps containing the solid oxidizing agent, hopcalite (a mixture of copper and manganese oxides; Hopkin and Williams Ltd., Chadwell Heath, Essex, England). The mercury content of traps was determined from the known specific activity of the vapor and by direct measurement of radioactivity using a γ-scintillation detector (Tracerlab). Samples and background were counted for a period of one hour each such that the counting error was normally less than 5%.

Leaf Temperature

An estimate of the water vapor concentration within leaves was derived from equation 22.3, knowing q_V, C_A and $r_{L.H_2O}$. An estimate of average leaf temperature was then derived from saturation vapor density tables by making the usual assumption that C_L is saturated at leaf temperature.

Plant Preparation, Exposure and Harvest

The plants for all experiments, which involved several species, were grown to a height of 15 cm in a glasshouse under natural light. The mean maximum and minimum daily temperatures were 26 and 19 C. Seeds were sown into free-draining 150 ml plastic pots containing a peat-loam soil mix. These were regularly watered with a 0.3% solution of a liquid fertilizer (10:5:5).

Fifteen hours prior to placement in the chamber, plants were thinned to a desired number, watered, and the soil surface sealed with petroleum jelly. Plants were then kept in the dark at room temperature (20 C) and allowed to drain into sand. Immediately prior to placement in the chamber, the pot was encased in a second pot which served to seal the base as well as provide the roots with mercury-free air. Plant transpiration and chamber temperature were then brought to a steady state over a period of 4.5 hours under the illumination conditions of the experimental run. These were normally of 5 hours duration, in which time 5 to 8 estimates of $r_{L.Hg}$ were made. After each exposure period, the chamber and plants were purged for 2 hours with mercury-free air. Plants were then kept overnight at 3 C and leaf area determined the following day. This was achieved by comparison of the mass of photo-copied replicates of the leaves with that of a template of known area. For harvest, plants were bulked into leaf, stem, and root components, and the radioactive mercury content determined for fresh and oven-dried material (24 hours at 80 C) in the same manner as were the traps.

Uptake of Mercury Vapor by Homogenized Leaves

Two grams of fresh leaves from young plants of several species were homogenized in 50 ml of 0.015 *M* phosphate buffer, pH 7.0, for one minute with an Omni-mixer. The container and buffer were pre-chilled to 0°C. Two ml of homogenate were pipetted into a test tube and a stream of air containing a known concentration of $^{203}\mathrm{Hg}^{0}$ vapor was passed through the suspension at a rate of 15 ml min^{-1} for a period of 15 minutes. The amount of ^{203}Hg uptake by the leaf homogenate was directly measured, corrected for the blank value using 2 ml of buffer alone, and calculated as percent conversion from the total ^{203}Hg bubbling through the system. The experiment was carried out either in light or darkness. A 75-watt reflector light bulb was used as the light source, and shined directly under a lucite water bath maintained at 25 C. The test tube was placed in the water bath about 8 cm from the light source which provided an illumination of 1.5 klux. When the experiment was carried out in the darkness, the test tube was wrapped with aluminum foil and the light was turned off. The ^{203}Hg in the homogenate was further analyzed for mercuric mercury.

Sorption of Mercury Vapor by Soil Materials

The radioactive mercury generator consisted of a 30 x 150 mm test tube with a sidearm outlet and an inlet tube at the top which extended almost to the bottom of the test tube. After transfer of the liquid ^{203}Hg (172 mg, 29.5 mCi) into the test tube, the generator was placed into a lead jar of 1-inch thickness to provide shielding, and the jar was immersed in a thermoregulated water bath. Air was pumped into the generator from the inlet tube at a constant flow rate which was measured by a precision flowmeter. The ^{203}Hg vapor was carried off from the generator and entered a mixing flask in which a mercury-free air was added to yield a desired concentration. The ^{203}Hg vapor concentration of the atmosphere was controlled by: 1) the temperature of the water bath; 2) the air flow rate into the generator; and 3) the dilution with mercury-free air. The determination of ^{203}Hg vapor concentration was achieved by passing a known volume of air through a hopcalite trap (a mixture of manganese oxide and copper oxide, Hopkin and Williams Ltd., Chadwell Heath, Essex, England), measuring the radioactivity in the trap, and calculating the vapor concentration from the known specific activity of the mercury. The temperature of water bath used in these experiments varied from 25 to 40 C.

In the experiments described here, a two gram sample of air-dry soil was placed in a test tube and exposed directly to a controlled atmosphere containing radioactive mercury vapor (^{203}Hg) for a given period of time and the uptake of ^{203}Hg vapor was determined by direct measurement of the radioactivity in the soil with a γ-scintillation spectrometer equipped with a 3-inch NaI well detector (Packard Model 5260 or Technical Associate Model SM-10). The background was generally less than 20 cpm for the Packard Model 5260 and 130 cpm for the SM-10, respectively. The ^{203}Hg metal was purchased commercially with an initial specific activity of 169 mCi/g mercury. The dry soils were exposed either for various lengths of time, or at different vapor concentrations. Samples of five soils from southeastern Montana, several clay minerals, sand, dry straw, humic acid, cellulose powder, peat, and charcoal were included in this study.

For the exposure of large amounts of soil, four 200 g samples of either Arvada, Heldt, Bainville or Terry, and 100 g of Campspass soil were spread individually in 10 cm diameter petri dishes which were randomly arranged in a sealed bell jar. The top of the bell jar contained an inlet and outlet for passing the air containing ^{203}Hg vapor through the bell jar. The inlet tube was at the upper part of the bell jar with a small orifice and bent outward, so as to create some mixing action. The outlet tube extended almost to the bottom of the bell jar. A magnetic fan inside the bell jar operated continuously to give additional mixing in order to avoid stratification of the mercury vapor. The flow-rate was maintained at 100 ml per minute. The concentration of mercury vapor was measured before and after entering the bell jar. The difference between these two measurements was due to the uptake by soil. These soil samples were exposed continuously for 23 days. The vapor concentration was measured daily except on the weekend. After exposure, the soil samples were removed from the bell jar and the radioactivity of each was measured using a 2 g sample. The soils were then subjected to the following examination in order to learn the nature and characteristics of the radioactive mercury retained by the soil. The treatments were: 1) 24 hours under vacuum (5 mm pressure); 2) 110 C heating for 2 hours; 3) Suspending 2 g soil in 10 ml of tris buffer containing 50 mg cysteine, bubbling air through the suspension for 30 minutes, and trapping the vapor (volatile mercury); and 4) Bubbling air through the soil suspension from (3) after addition of stannous chloride solution and trapping the vapor (mercuric mercury).

To determine whether or not the sorbed mercury was available for plant uptake, 50 wheat seeds were sown in each soil (200 g) and allowed to germinate under greenhouse conditions. The soils were maintained moist by adding water as needed. After 48 and 72 hours, ten seeds or seedlings were carefully removed from each soil, washed thoroughly to remove any soil particles and their radioactivity determined. On the 7th and 10th days, the tops of ten plants were cut off and counted. Because of the low level of radioactivity observed, each sample was counted for 60 minutes to ensure the accuracy of counting. After the wheat cultivation, the soils were allowed to dry at room temperature, and 2 g aliquots were taken from each sample for the measurement of total and mercuric mercury as described previously.

RESULTS AND DISCUSSION

Estimation of Whole-Plant Resistance to Gaseous Exchange

The efficacy of estimating $r_{L.H_2O}$ on a whole-plant basis by the induction of small changes in ambient humidity was examined in three initial experiments. The study was confined to wheat plants (*Triticum aestivum*) which were 11 days of age from sowing.

Magnitude of Humidity Change Versus $r_{L.H_2O}$

The term $r_{L.H_2O}$ was estimated for a group of 12 wheat plants by decreasing the relative vapor pressure of incoming air by progressively larger increments. After each incremental decrease, the inlet relative vapor pressure, or relative humidity, was approximately returned to the initial level of 0.47. Total leaf surface was 154 cm^2, the illumination 6.4 klux, and the mean chamber temperature 32.90 ± 0.05 C.

The relationship between change in inlet and outlet relative vapor pressures is shown in Figure 22.3. The linearity of the relationship is indicative of the plants remaining in a stable state during the course of the experiment, irrespective of the direction or magnitude of change in humidity. The considerable influence of the plants upon humidity within the chamber is evident from the slope of the regression equation, and also the mean ambient relative vapor pressure of 0.79. The slope of the function would tend towards unity with increases in leaf resistance and chamber flow rate, or a decrease in leaf surface area. Optimization of the quantity of plant material placed in the chamber was therefore necessary to ensure that changes in outlet humidity could be adequately resolved, and at the same time avoid the creation of excessively high evaporative conditions.

From each change in humidity in Figure 22.3, an estimate of $r_{L.H_2O}$ was derived using equation 22.7, and these values are presented in Figure 22.4A. The mean $r_{L.H_2O}$ (± SD) was 3.5 ± 0.4 sec cm^{-1}. The correlation ($r = 0.32$) between magnitude of change in inlet relative vapor pressure and estimated $r_{L.H_2O}$ was not significant ($P = 0.05$). In other words, changes in ambient or outlet relative vapor pressure ranging from 0.019 to 0.074 (Figure 22.3) caused no significant change in estimates of $r_{L.H_2O}$. The implication is that estimates of $r_{L.H_2O}$ did not substantially differ from the situation where $\Delta e/e_o$ (inlet) $\rightarrow 0$ and where errors arising from the assumptions concerning $r_{L.H_2O}$ and leaf temperature (T_L) would be minimal. This was an encouraging result since it indicates that Jarvis' approach is applicable over an operable range of induced humidity changes.

The reason for such a result could not be determined from our data since our facilities did not permit detection of small changes in leaf temperature. One possibility is that the theory and assumptions, as

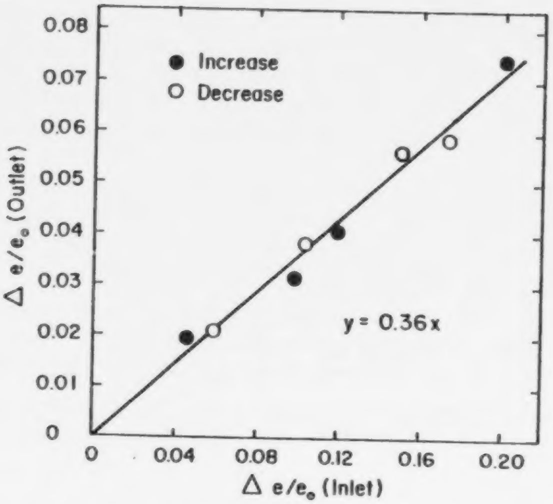


Figure 22.3. Relationship between induced changes in relative vapor pressure (e/e_o) of air entering and exiting the plant chamber. The chamber contained a group of 12 wheat plants at a temperature of 39.2 C and an illumination of 6.4 klux.

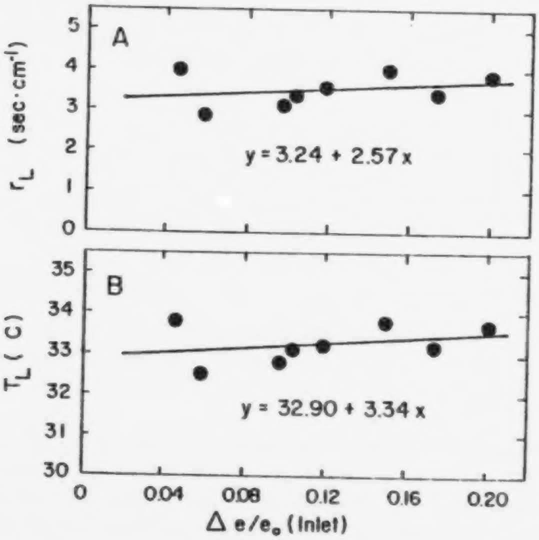


Figure 22.4. Relationship between induced changes in relative vapor pressure (e/e_o) of air entering the plant chamber and estimates of : (A) total leaf resistance to water vapor exchange (r_L) and (B) leaf temperature (T_L). The chamber contained a group of 12 wheat plants at a temperature of 32.9 C and an illumination of 6.4 klux.

proposed, are correct and that the term $\Delta C_L/\Delta q_v$ (equation 22.6) was in fact small or negligible. The mean value of Δq_v ($\Delta\bar{q}_v$) in this experiment was 0.44×10^{-6} g cm^{-2} sec^{-1}. If over the range of changes in q_v, leaf temperature changed by ± 0.1 C about a mean, then the value of $\Delta C_L/\Delta\bar{q}_v$ would have been ± 0.4 sec cm^{-1} where $T_L \sim 33$ C and $\Delta C_L = \pm 0.19 \times 10^{-6}$ g cm^{-3}. This corresponds closely to the range of values determined for $r_{L.H_2O}$ (Figure 22.4A).

A second possibility is that leaf temperature did change, but that ΔC_L was proportional to Δq_v such that the neglected term of equation 22.6 represented a constant fraction of $\Delta C_A/\Delta q_v$. Yet a third possibility is that both leaf temperature and $r_{L.H_2O}$ changed by some increment. There is evidence for a number of species that stomatal resistance does in fact increase with increasing deficit between leaf and ambient water vapor concentration (Aston, 1976; Hall and Kaufmann, 1975). Simultaneous changes in both $r_{L.H_2O}$ and leaf temperature, however, could be mutually exclusive with respect to effect on transpiration (Gates, 1968). If such were the case, then $\Delta C_A/\Delta q_v$ would effectively be a function of $r_{L.H_2O}$ prior to the induced change in C_A.

From these alternatives, it is obvious that the theory, as proposed by Jarvis (1971), is worthy of a more exhaustive examination. Short of this, the indirect evidence of Figure 22.4A is that the approach does provide a reasonable estimate of the real value of $r_{L.H_2O}$. On this basis, determined values of $r_{L.H_2O}$ were substituted into equation 22.3 to derive estimates of average leaf temperatures, as described in the methods section. These estimates are presented in Figure 22.4B as a function of change in inlet relative vapor pressure. The mean leaf temperature of 33.3 ± 0.5 C was close to the ambient temperature of 32.9 C.

Humidity Change and Temperature Versus $r_{L.H_2O}$

Total leaf resistance of three groups of 15 wheat plants was determined in separate experimental runs at ambient temperatures of 17, 25, and 33 C. Illumination was 6.4 klux and the respective leaf surface areas were 185, 180, and 174 cm^2. Estimates of $r_{L.H_2O}$ were derived by progressively decreasing inlet relative vapor pressure by increments of 0.05, 0.10, and 0.15. After each decrease, relative vapor pressure of the incoming air was returned to an initial level of 0.42.

The results of this experiment support those results of the previous experiment in that neither magnitude nor direction of change in inlet humidity had significant effect ($\rho = 0.05$) upon the estimated value of $r_{L.H_2O}$ (Table 22.2). There was no effect of ambient temperature, and there were no obvious interactions. Change in outlet relative vapor pressure ranged from 0.011 to 0.044. This was less than the comparable range in the previous experiment (Figure 22.3), and was due to either the increased leaf surface areas, lower leaf resistances, or both. Less variable estimates of

---

$r_{L.H_2O}$ were obtained with more substantial changes in humidity and also higher temperatures (Table 22.2). This was a result of increased wet bulb depression and hence reduced resolution errors in psychrometry.

TABLE 22.2. TOTAL LEAF RESISTANCE VERSUS CHANGE IN RELATIVE VAPOR PRESSURE (e/e_o) OF AIR ENTERING THE CHAMBER

		Temperature (C)			
$\Delta e/e_o$	Direction	17	25	33	Mean (SE)
			sec cm^{-1}		
0.05	-	2.8	2.1	3.2	3.0 (0.3)
0.05	+	4.0	3.0	3.1	
0.10	-	3.3	2.8	2.7	2.8 (0.2)
0.10	+	2.1	2.8	2.9	
0.15	-	3.2	2.9	2.7	2.8 (0.1)
0.15	+	2.3	2.9	2.7	
	Mean (SE)	3.0 (0.3)	2.8 (0.1)	2.9 (0.1)	General Mean 2.9

The lack of response of leaf resistance to temperature is notable since these temperatures encompassed most of the range favorable to growth. Apparently humidity levels within the chamber were sufficiently high to eliminate indirect temperature effects. Slatyer and Bierhuizen (1964) reported no effect of temperature on stomatal resistance of cotton leaves in the range from 30 C to 40 C, while Hsiao (1975) concluded from the limited data available that the slope of stomatal response to temperature curves is gentle within the range of favorable growing temperatures.

Illumination and Temperature Versus $r_{L.H_2O}$

Total leaf resistance values were determined for groups of 15 wheat plants illuminated at 0, 1.6, 3.2, 6.4, or 12.8 klux at ambient temperatures of 17, 25, or 33 C. Mean leaf area was 190 ± 25 cm^2. Five determinations of $r_{L.H_2O}$ were made during a five-hour period by alternately decreasing and elevating vapor pressure of incoming air by an increment of 0.10. Relative vapor pressure of air entering the chamber prior to the induced decrease was 0.42.

For convenience of scale, the response to illumination of leaf conductance ($r_{L.H_2O}^{-1}$), rather than leaf resistance, is presented in Figure 22.5. Response at each temperature is indicated separately, although a second order polynomial was fitted to the combined data by regression techniques, the equation being

$$Y = (1.8 \times 10^{-3}) + (7.2 \times 10^{-2})\ X - (3.0 \times 10^{-3})\ X^2 \qquad (22.10)$$

While other forms may have been more appropriate (Osman and Milthrope, 1971), this equation accounted for 94% of variation in leaf conductance, and indicates negligible temperature effect within the examined ranges, as well as a maximum value for $r_{L.H_2O}^{-1}$ of 0.43 at an illumination of 12.0 klux.

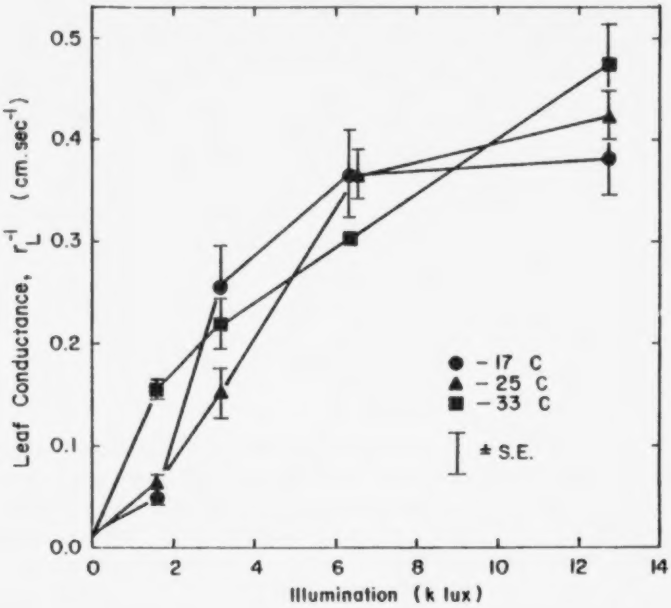


Figure 22.5. Effect of illumination on estimated leaf conductance of water vapor (r_L^{-1}) for groups of wheat plants at 3 ambient temperatures. Each estimate is the mean of 5 determinations during a 5 hour exposure period.

Theoretical estimates of leaf temperature were derived in this experiment as described under Materials and Methods, and the deviations from ambient temperature are presented in Table 22.3. Except in darkness, the tendency for leaf temperature to exceed ambient temperature became less as leaf resistance decreased and ambient temperature increased. At the lowest resistance values, and in darkness, leaf temperature tended to be below ambient temperature. These trends are in agreement with those theoretically computed by Gates (1968), although the radiant conditions here were not constant.

The functional nature of this technique for estimating $r_{L.H_2O}$ is demonstrated by the wide range of conditions over which estimates were derived. The approach is attractive for its simplicity and shares a number of other advantages with steady-state diffusion porometers (Campbell, 1975), in addition to being applicable to whole-plant studies. The method was therefore adopted for the purposes of this work, although further examination of the unresolved aspects of the theory appears warranted.

TABLE 22.3. ESTIMATED DIFFERENCE BETWEEN LEAF AND AMBIENT TEMPERATURE

	Difference (C)				
17	-1.9[a] (1.7)	9.1 (2.6)	1.1 (1.1)	0.8 (0.4)	0.7 (0.4)
25	0.0 (1.8)	5.9 (1.9)	2.9 (0.9)	0.3 (0.2)	-0.4 (0.2)
33	-1.7 (1.3)	2.0 (0.4)	1.3 (0.5)	0.4 (0.1)	-0.7 (0.3)
	sec cm^{-1}				
Total leaf resistance	82.9[b] (5.1)	16.7 (6.2)	5.6 (0.8)	3.0 (0.2)	2.4 (0.2)

[a] Mean (SE) of 5 estimates derived over a period of 5 hours.

[b] Mean (SE) for all ambient temperatures.

Factors Influencing the Uptake of Hg Vapor by Wheat

In a second series of experiments, selected environmental parameters were examined in relation to their effect on components of the proposed model for mercury vapor uptake (equation 22.1). The study was again confined to 11 day-old wheat plants and the factors examined were ambient temperature, ambient mercury vapor concentration, illumination, and duration of exposure.

Illumination and Temperature Versus Hg Uptake

Groups of 15 plants were exposed to a mean mercury vapor concentration of 16 (± 2) $\mu g\ m^{-3}$ for a duration of 5 hours at illuminations of 0, 1.6, 3.2, 6.4, or 12.8 klux. Similar exposures were repeated at ambient temperatures of 17, 25, and 33 C. Distribution of mercury accumulated by the plants is shown in Table 22.4. The incorporated mercury was largely immobile, as indicated by the almost exclusive confinement to leaves. Oven drying (80 C) of the leaves released a mean 10% of the mercury, with no detectable changes in the mercury content of stems and roots.

Both stomatal and biochemical regulation of mercury vapor uptake might be inferred from the pronounced decrease in dark uptake (Table 22.4). This can be further appreciated from Figure 22.6, where the effect of both illumination and ambient temperature on mercury uptake by leaves is depicted. Maximum uptake at each ambient temperature was attained at the low illumination level of 3.2 klux.

TABLE 22.4. DISTRIBUTION OF MERCURY ACCUMULATED BY WHEAT. PLANTS EXPOSED TO MERCURY VAPOR (16 μg m^{-3}) FOR 5 HOURS

Site	Illumination (klux)				
	0	1.6	3.2	6.4	12.8
			ng/plant		
Leaves	9(2)[a]	38(6)	51(13)	60(8)	56(7)
Stems	0(0)	1(1)	2(1)	2(2)	1(1)
Roots	0(0)	1(1)	2(1)	2(1)	0(0)

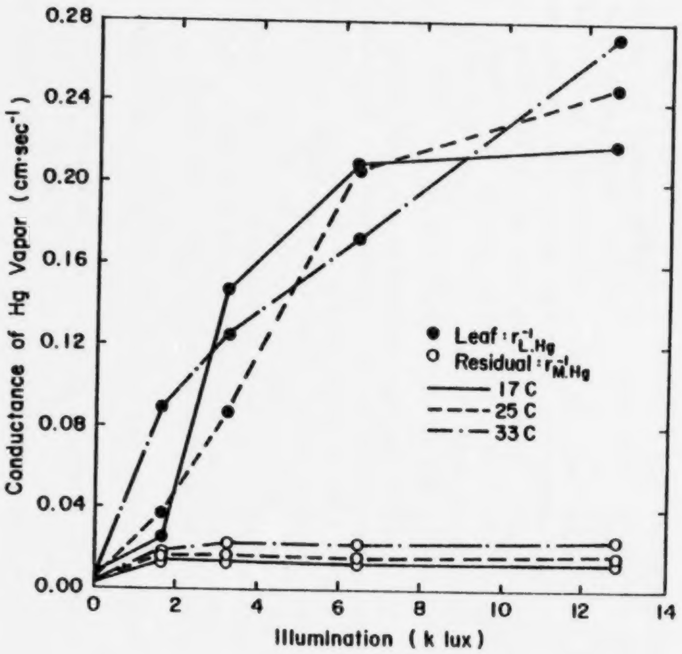
[a] Mean uptake (SE) by fresh material at ambient temperatures of 17, 25, and 33 C.

Conductance and Resistance to Hg Uptake

During each 5 hour exposure period in this experiment, five estimates of leaf conductance of mercury vapor ($r_{L.Hg}^{-1}$) were made. The reciprocal of the mean value of $r_{L.Hg}^{-1}$ was then taken as the average value of $r_{L.Hg}$ for the same period. This was considered to provide a better estimate of the value of $r_{L.Hg}$ since gaseous exchange is a direct function of conductance rather than resistance. Substitution of this value into equation 22.1, together with determined values for $C_{A.Hg}$ and mercury uptake, then generated a value for the residual conductance of mercury vapor ($r_{M.Hg}^{-1}$). The effect of illumination and ambient temperature on $r_{L.Hg}^{-1}$ and $r_{M.Hg}^{-1}$ is shown in Figure 22.7. The parameter $r_{L.Hg}^{-1}$, for which stomata represent the major variable, was highly influenced by illumination level; however, no effect of temperature on $r_{L.Hg}^{-1}$ was discernible from these results.

Since $r_{L.Hg}$ and $r_{M.Hg}$ are considered to be in series, it can be appreciated that the low value of $r_{M.Hg}^{-1}$ (Figure 22.7) was the predominant factor limiting mercury uptake. The term $r_{M.Hg}^{-1}$ (Figure 22.7) was largely insensitive to illumination levels other than darkness, and this accounts for the rapid plateauing of mercury uptake seen in Figure 22.6. The term $r_{M.Hg}^{-1}$ was influenced by temperature, although this is not obvious in Figure 22.7. This is apparent in Figure 22.8, however, where $r_{M.Hg}$ has been expressed as a linear function of leaf temperature for all illumination levels other than darkness. The plot of $r_{M.Hg}$ versus leaf, rather than ambient, temperature is a recognition of the biochemical component of $r_{M.Hg}$. Leaf temperature, however, only substantially differed from ambient temperature at the low illumination level of 1.6 klux. Thus a linear regression of $r_{M.Hg}$ versus ambient temperature would have equally accounted for the variation in $r_{M.Hg}$ ($r^2 = 0.88$), with only a slight change in slope ($b = -1.9$) and position ($a = 111.2$). Given a value for $r_{M.Hg}$ of 117.7-2.0x (Figure 22.8), it can be seen that $r_{M.Hg}^{-1}$ would increase at an increasing rate with temperature. Hence, in Figure 22.7, the difference in mercury uptake in light between 25 and 33 C was greater than that between 17 and 25 C.

Figure 22.7. Effect of illumination and ambient temperature on leaf ($r^{-1}_{L.Hg}$) and residual conductances of mercury vapor ($r^{-1}_{M.Hg}$). Each point is the mean for a group of 15 wheat plants exposed to mercury vapor (16 μg m^{-3}) for a period of 5 hours.



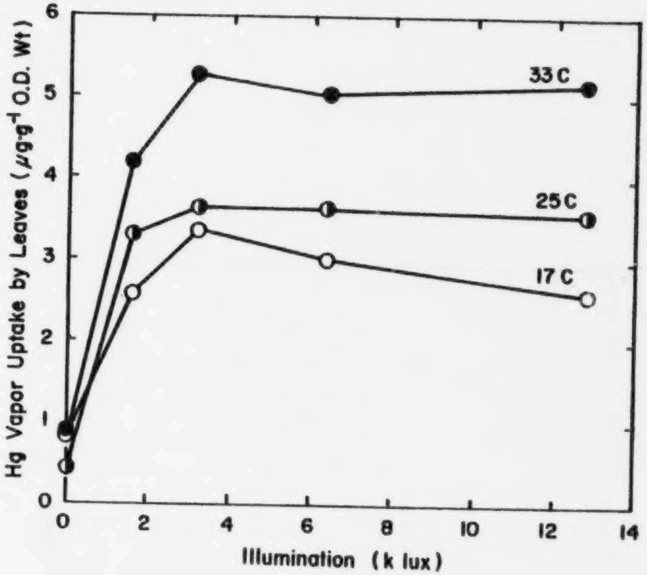


Figure 22.6. Effect of illumination and ambient temperature on mercury uptake by leaves of wheat plants exposed to mercury vapor (16 μg m^{-3}) for 5 hours.

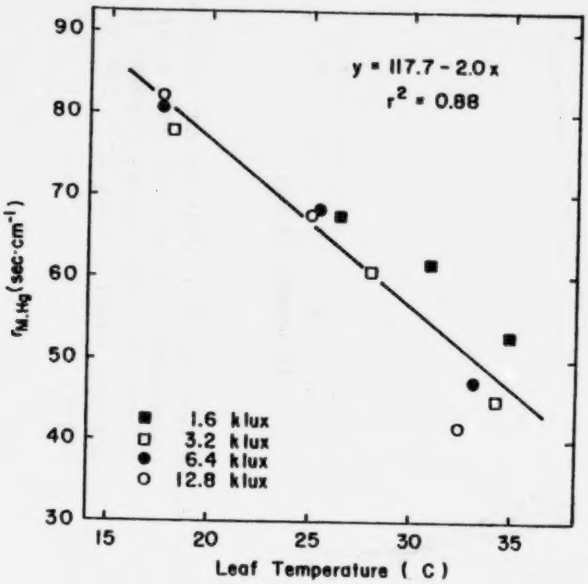


Figure 22.8. Effect of leaf temperature on the residual resistance to mercury vapor uptake ($r_{M.Hg}$) in light (experiment 4).

The apparent low values of $r_{M.Hg}{}^{-1}$ in darkness (Figure 22.7) were in contrast to those in light, and corresponded to the high $r_{M.Hg}$ values of 237, 488, and 277 sec cm^{-1} at ambient temperatures of 17, 25, and 33 C. These high values may, in part, have been a result of the inappropriate nature of the assumption that the diffusion coefficients were independent of stomatal aperture. With incomplete molecular slip, the reduction in diffusion coefficients could be appreciable in the narrowest regions of stomatal opening (Milthorpe and Penman, 1967; Cowan and Milthrope, 1968). If the reduction in D^o_{Hg} was greater than for $D^o_{H_2O}$, then $r_{L.Hg}$ as derived from equation 22.2 would be an underestimate, and hence the residual value of $r_{M.Hg}$ from equation 22.1, an overestimate. Alternatively, or concurrently, there may have been a true increase in $r_{M.Hg}$, reflecting change in biochemical resistances in the dark. This may have been accentuated by the prolonged period in which plants were prepared in darkness. Such questions have not been resolved in this study and are worthy of further investigation. The high values for $r_{M.Hg}$ in darkness do indicate, however, that the mercury binding to external surfaces, if any, was small.

Vapor Concentration and Temperature Versus Hg Uptake

Groups of 15 wheat plants were exposed to mercury vapor concentrations of 0, 1.6 (±0.2), 16 (±2), or 40 (±5) μg m^{-3} at an illumination of 6.4 klux. The exposures were replicated at ambient temperatures of 17, 25, and 33 C.

The durations of exposure were 5 hours, in which time 6 to 8 estimates of $r_{L.Hg}^{-1}$ were made. Average values of $r_{L.Hg}$, which are presented in Table 22.5, were derived from the mean value of $r_{L.Hg}^{-1}$. Analysis of variance revealed no effect of mercury vapor concentration nor ambient temperature on $r_{L.Hg}$ at the 5% level of significance. There was no apparent interaction.

TABLE 22.5. EFFECT OF AMBIENT Hg VAPOR CONCENTRATION AND AMBIENT TEMPERATURE ON TOTAL LEAF RESISTANCE TO Hg VAPOR EXCHANGE ($r_{L.Hg}$)*

Hg Vapor Concentration ($\mu g\ m^{-3}$)	Ambient Temperature (C)			
	17	25	33	Mean
		$r_{L.Hg}$ (sec cm^{-1})		
0	4.9	4.7	5.0	4.9
1.6	5.4	5.6	6.1	5.7
16	4.8	4.8	5.8	5.1
40	4.1	5.3	5.3	4.9
Mean	4.8	5.1	5.6	5.2

*Illumination was 6.4 klux.

The $r_{M.Hg}$ values were determined as in the previous experiment and these are presented as a function of leaf temperature in Figure 22.9. The results reaffirm the linearity of the relationship seen in Figure 22.8. Apart from the obvious effect of temperature, there was no significant effect of mercury vapor concentration on $r_{M.Hg}$ (5% level). This result is supported by the data in Figure 22.10, where the rate of mercury uptake by leaves is depicted as a function of ambient mercury vapor concentration. The curves for each ambient temperature are essentially linear and pass through the origin. Given that $r_{L.Hg}$ was effectively constant (Table 22.5), any deviation of the curves from linearity would have indicated changes in $r_{M.Hg}$ or $C_{L.Hg}$ (equation 22.1). As this was not the case, the assumption that $C_{L.Hg}$ was zero appears to have been justified in these experiments. If the binding sites for mercury vapor were specific, then this assumption could become less valid under conditions inducive to high accumulation of mercury.

The mercury vapor concentrations examined in these experiments were high when compared to normal background levels, which may range from 0.001 to 50 ng m^{-3} (D'Itri, 1972). High atmospheric concentrations have been reported, however, such as 18 $\mu g\ m^{-3}$ in an industrialized area of Japan (Fujimura, 1964) and up to 40 $\mu g\ m^{-3}$ in thermal areas of the Hawaiian Islands (Siegel and Siegel, 1975).

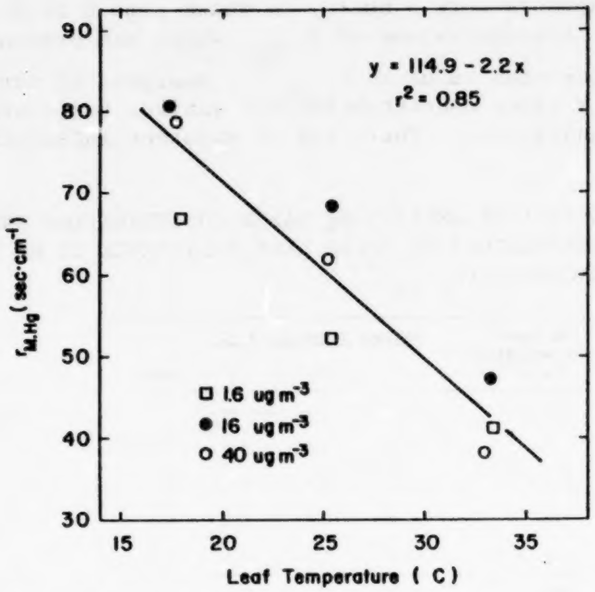


Figure 22.9. Effect of leaf temperature on the residual resistance to mercury vapor uptake ($r_{M.Hg}$) at 3 ambient mercury vapor concentrations. Illumination was 6.4 klux and the exposure period 5 hours.

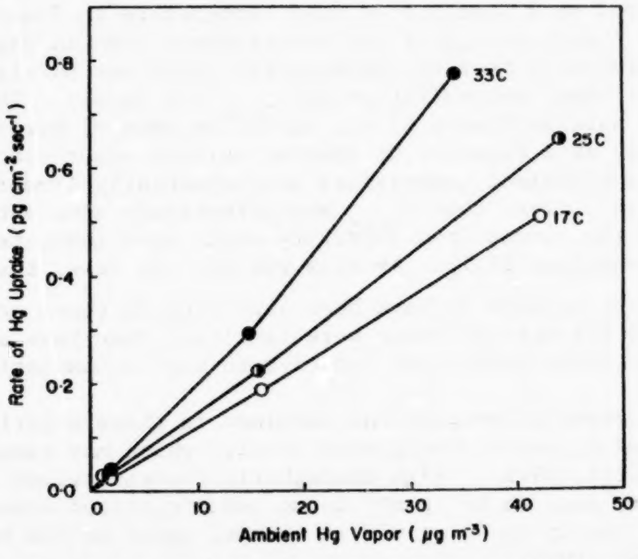


Figure 22.10. Relationship between ambient mercury vapor concentration and the rate of mercury vapor uptake by wheat leaves at 3 ambient temperatures and an illumination of 6.4 klux.

Duration of Exposure Versus Hg Uptake

Groups of 15 wheat plants were exposed to a mean mercury vapor concentration of 1.01 ($\pm$0.08) $\mu g\ m^{-3}$ for periods of 2, 4, 6, and 8 hours. The illumination was 6.4 klux and the ambient temperature was 25 C. Mercury vapor uptake by leaves was determined and was found to be linearly related to the duration of exposure (Figure 22.11). These results correspond closely to those of the previous experiment, as demonstrated by the comparison in Figure 22.11 of actual mercury vapor uptake with that predicted from equation 22.1 knowing $C_{A.Hg}$ and predicting $r_{L.Hg}$ from Table 22.5 and $r_{M.Hg}$ from Figure 22.9. The linearity of this relationship and of those in Figure 22.10 indicates that mercury vapor uptake in these experiments did not approach limiting levels.

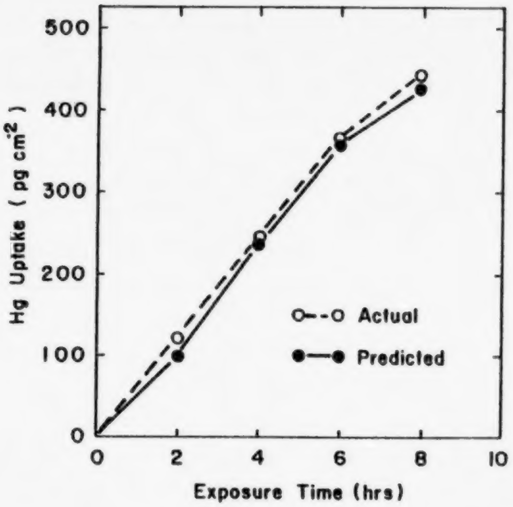


Figure 22.11. The relationship between duration of exposure and mercury uptake (actual and predicted) by leaves of wheat plants exposed to mercury vapor (1.0 $\mu g\ m^{-3}$) at 25 C and an illumination of 6.4 klux.

The close association between $r_{M.Hg}$ and temperature, which prevailed in previous experiments is intriguing. This suggests that $r_{M.Hg}$ does substantially reflect the rate of biochemical reactions involved in the binding of mercury vapor. Although the nature of $r_{M.Hg}$ at this point remains uncharacterized, it can be said that the model was successful in partitioning the effect on mercury vapor uptake of those parameters which were studied.

Differential Uptake of Hg Vapor by Plants

Mercury vapor uptake by six plant species was examined under uniform conditions. The selected species all belonged to the family Gramimeae since their growth habit was suited to the determination of resistances to gaseous exchange using the present theory and plant chamber. The species were oats (*Avena sativa*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), corn (*Zea mays*), sorghum (*Sorghum vulgare*), and crabgrass (*Digitaria sanguinalis*). Oats, barley and wheat are plants characteristically possessing the C_3 photosynthetic carbon fixation pathway, while corn, sorghum, and crabgrass are C_4-pathway plants.

Pots containing several plants of the one species were exposed for a period of 4 hours to a mercury vapor concentration of 4.4 (±0.3) μg m^{-3}. The illumination was 11 klux and the ambient temperature was 25 C. The experiment was replicated three times and was of a completely randomized design. The optimum height and leaf surface area of plant material with regard to the plant chamber was 15 cm and 250 cm^2 respectively. The age and number of plants constituting an exposed group therefore differed between species. The manner in which these prerequisites was met is presented in Table 22.6.

TABLE 22.6. PARTICULARS OF PLANT SPECIES EXPOSED TO Hg VAPOR

Species	Plants/pot	Mean leaf surface area (cm^2)	Age (days from sowing)
Oats	20	255 (16)[a]	8
Barley	17	278 (29)	8
Wheat	18	231 (23)	11
Corn	6	281 (14)	12
Sorghum	10	192 (26)	11
Crabgrass	11	267 (13)	19

[a] Mean (SD)

Mercury vapor uptake by the plants was determined and the results are presented in Table 22.7. As in previous experiments, mercury vapor in all species was seen to accumulate predominantly in the leaves. The mercury uptake by leaves differed between species, but the pronounced difference was that which existed between C_3 and C_4-type plants. The reason for this difference is apparent in Table 22.8, where the calculated values for components of the model for mercury uptake (equation 22.1) are presented. The difference in Table 22.8 between $r_{L.Hg}$ values for C_3 and C_4 plants would be expected in view of the difference in light saturation characteristics. The effect of $r_{L.Hg}$ on mercury uptake was small, however, compared to that of $r_{M.Hg}$, the values of which were high in C_4 plants. Such differences cannot be reasonably attributed to leaf age (Table 22.6) nor leaf temperature

TABLE 22.7. TOTAL Hg UPTAKE BY PLANTS

Species	Hg uptake (ng)		
	Leaves	Stems	Roots
Oats	198 (a)[*]	2.3 (c)	0.9 (a)
Barley	226 (b)	1.4 (c)	1.6 (b)
Wheat	201 (a)	5.0 (ab)	0.9 (a)
Corn	40 (cd)	3.5 (bc)	0.6 (a)
Sorghum	25 (d)	2.6 (bc)	0.3 (a)
Crabgrass	54 (c)	6.5 (a)	0.7 (a)
SE	7	0.8	0.2
F ratio	183	5.4	4.7
Significance level	<0.001	<0.01	<0.05

[*] Means followed by a common letter do not differ at a significant level of 5% (DMR test).

TABLE 22.8. A SUMMARY OF RESISTANCES TO Hg VAPOR UPTAKE BY PLANT SPECIES

Species	$r_{L.Hg}$ (sec cm^{-1})	$r_{M.Hg}$ (sec cm^{-1})	T_L (C)
Oats	4.9 (a)[*]	72.0 (a)	24.9 (a)
Barley	5.9 (a)	65.4 (a)	25.0 (a)
Wheat	4.0 (a)	60.1 (a)	24.8 (a)
Corn	14.2 (b)	399.4 (c)	26.2 (b)
Sorghum	23.2 (c)	448.9 (d)	27.9 (c)
Crabgrass	13.9 (b)	305.4 (b)	26.2 (b)
SE	1.0	19.4	0.3
F ratio	52.9	86.3	20.9
Significance Level	<0.001	<0.001	<0.001

[*] Means followed by a common letter do not differ at a significance level of 5% (DMR test).

(Table 22.8), and must reflect true differences in biochemical and physical resistances to mercury vapor uptake ($r_{M.Hg}$) between the examined C_3 and C_4 species. Mercury is known to inhibit photosynthetic processes (Horwitz, 1957; Bradeen and Winget, 1974), and this apparent affinity of the vapor for C_3-type plants indicates that the binding sites for the vapor may be specific. As such, mercury vapor uptake could be a useful tool for photosynthetic studies.

In Vitro Conversion of Elemental Mercury Vapor by Homogenized Leaves of Various Plant Species

In the experiments with wheat plants so far, the major factor governing mercury vapor uptake, besides ambient vapor concentration, has been shown to be leaf temperature. In terms of the model used, the effect of temperature can be explained by its influence on those internal leaf resistances to vapor uptake encompassed by the term $r_{M.Hg}$. Values of $r_{M.Hg}$ were found to decrease with increasing leaf temperature and, except for high values in darkness, the relationship was linear and independent of illumination level and mercury vapor concentration over the ranges examined.

Differences found in mercury vapor uptake by C_3 and C_4 plant species under uniform conditions were also largely attributable to differences in the value of $r_{M.Hg}$. Since, by definition, biochemical processes contribute to the value of $r_{M.Hg}$, the following experiments were conducted with homogenized leaves with a view to elucidating the possible nature and sites of the processes involved. Several plant species were examined and these included the C_3 species - wheat, barley, oats, and water spinach; and the C_4 species - corn, sorghum, and crabgrass. Leaf homogenates were prepared and exposed to mercury vapor as described under Materials and Methods.

Due to the destruction of cellular integrity by the homogenizing procedure, mercury vapor uptake by the homogenates can be expected to be far less specific than in the case of the whole plant. There was, however, notable agreement between results of these experiments and those involving whole plants in that the conversion of mercury vapor was greater in light than in darkness for all species except sorghum (Table 22.9). Homogenates of all C_3 plants also exhibited higher percent conversion values than those of C_4 plants, both in light and in darkness (Table 22.9). The rate of conversion of mercury vapor by the homogenates was linearly related to vapor concentration (Figures 22.12-22.15), as was also found with whole wheat plants (Figure 22.10). Higher vapor concentrations resulted in greater mercury uptake, although the homogenates of younger leaves of wheat and crabgrass tended to have higher percent conversion values, possibly owing to higher enzymatic activity.

Boiling the leaf homogenate resulted in a 90 per cent loss of activity. Enzyme inhibitors such as sodium azide, sodium cyanide, sodium fluoride, sodium arsenite, and sodium malonate showed various degrees of inhibition (Table 22.10). The specific photosynthetic inhibitor monuron gave a marked inhibition of mercury vapor conversion in the case of water spinach. Amitrole, an inhibitor of catalase, also gave a high inhibition. Such results indicate that the conversion and uptake of elemental mercury vapor by plant

TABLE 22.9. *IN VITRO* CONVERSION OF $^{203}Hg^{o}$ VAPOR BY THE LEAF HOMOGENATE OF VARIOUS PLANT SPECIES

Species	% Conversion		Light/Dark
	Light	Dark	
Water spinach	20.6 ± 4.4 (12)	8.6 ± 2.8 (12)	2.4
Wheat	11.0 ± 6.4 (8)	5.2 ± 3.6 (8)	2.1
Barley	12.2 ± 1.6 (4)	7.9 ± 1.5 (4)	1.5
Oat	12.3 ± 1.5 (4)	8.6 ± 1.2 (4)	1.4
Corn	3.1 ± 0.7 (6)	2.2 ± 0.9 (6)	1.4
Crabgrass	7.9 ± 4.3 (11)	4.4 ± 2.8 (11)	1.8
Sorghum	1.1 ± 0.3 (4)	2.2 ± 0.2 (4)	0.5

2 ml of leaf homogenate (0.08 g fresh leaf) were used in each experiment. Air containing different $^{203}Hg^{o}$ vapor concentration was bubbled through the suspension for 15 minutes at 15 ml per minute. Percent conversion was calculated from the net uptake by the leaf homogenate and the total amount of mercury in the air which passed through the homogenate.

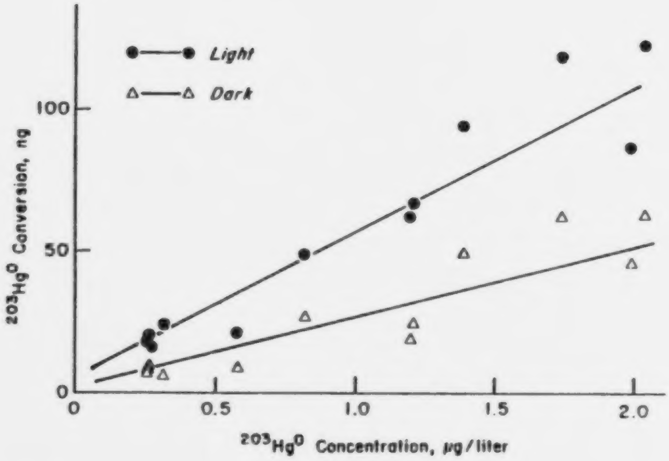


Figure 22.12. *In vitro* conversion of ^{203}Hg vapor by water spinach leaf homogenate in relation to the vapor concentration. Homogenate used = 0.08 g fresh leaf; time = 15 min.

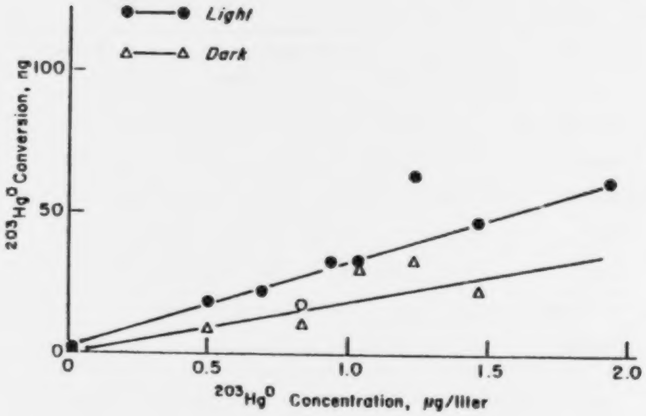


Figure 22.13. *In vitro* conversion of ^{203}Hg vapor by wheat leaf homogenate in relation to the vapor concentration. Homogenate used = 0.08 g fresh leaf; time = 15 min.

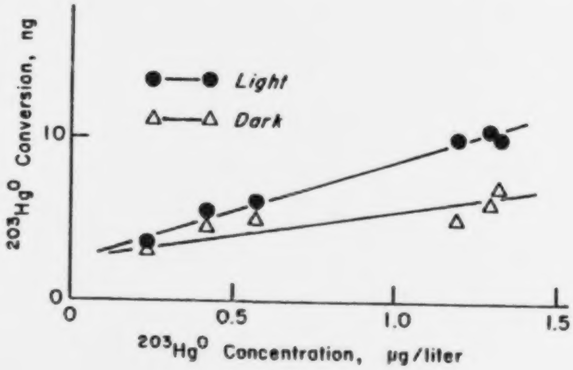


Figure 22.14. *In vitro* conversion of ^{203}Hg vapor by corn leaf homogenate in relation to the vapor concentration. Homogenate used = 0.08 g fresh leaf; time = 15 min.

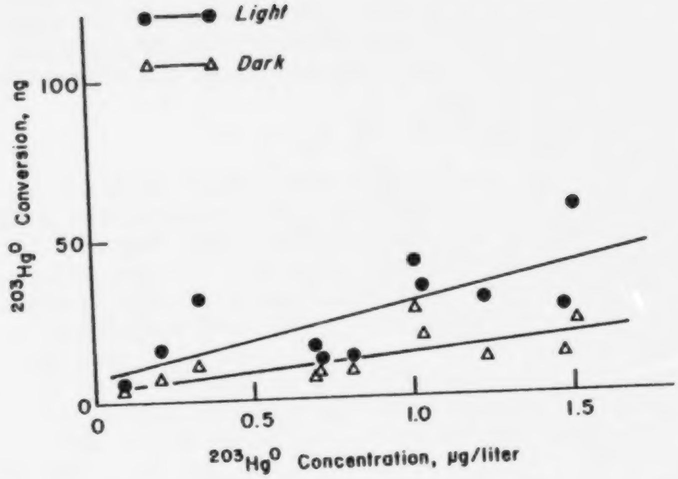


Figure 22.15. *In vitro* conversion of ^{203}Hg vapor by crabgrass leaf homogenate in relation to the vapor concentration. Homogenate used = 0.08 g fresh leaf; time = 15 min.

TABLE 22.10. INHIBITION OF ^{203}Hg VAPOR UPTAKE BY LEAF HOMOGENATE OF SOME C_3 AND C_4 PLANT SPECIES BY ENZYME INHIBITORS

Inhibitors		Water spinach		Wheat		Corn		Crabgrass	
		Light	Dark	Light	Dark	Light	Dark	Light	Dark
		%	%	%	%	%	%	%	%
NaN_3	1×10^{-3}M	82	91	100	100	92	100	84	100
NaF	1×10^{-3}M	17	0	36	39	47	57	25	25
$NaAsO_2$	1×10^{-3}M	26	58	30	82	26	40	8	54
NaCN	1×10^{-3}M	18	0	95	100	90	83	81	100
Melonate	1×10^{-3}M	48	31	18	50	35	30	9	28
Amitrole	1×10^{-3}M	75	77	95	100	48	69	40	46
Monuron	1×10^{-5}M	76	92	-	-	-	-	-	-

leaves is an enzymatically controlled process which is in part, at least, light dependent. A non-specific oxidative enzyme such as catalase may also be involved in such conversion, since greater than 90 per cent of the mercury in the leaf homogenates was in the mercuric form in all species examined.

Sorption of Mercury Vapor by Soils

Table 22.11 shows the result of mercury vapor sorption by the soils, clay minerals, peat, charcoal, sand, and organic materials which were exposed to an atmosphere containing 75.9 μg metallic ^{203}Hg vapor/m^3 for 24 hours at room temperature. Among the five soil samples, Campspass, which had the highest organic matter content, had the highest uptake, followed closely by the Heldt and Bainville soils. The high clay content Arvada and sandy Terry soils sorbed approximately one-fourth of the amount sorbed by the Campspass soil. The peat sample sorbed about twice the amount of Hg as the Campspass soil. Humic acid sorbed slightly more Hg than the peat. Granular charcoal sorbed about twenty times as much Hg as the peat, while the dry straw and cellulose powder sorbed very little Hg. Among the clay minerals, illite had the highest sorbing power (0.308 μg) for elemental mercury vapor, while kaolinite had the lowest (0.004 μg). Higher sorption of Hg^o vapor by illite as compared to montmorillonite and kaolinite was also reported by Trost and Bisque (1972). The nature of organic matter in the soil was also important, as humic acid sorbed a great deal more mercury vapor than dry straw or cellulose powder. The organic-rich A soil horizons studied by Trost and Bisque (1972) all sorbed more vaporous mercury than did the clay-rich B soil horizons, and they suggested that the variations in mercury sorbed by the A horizons may reflect chemical variations in the type of humic matter forming under different vegetative covers. The wide range of sorption observed here with these materials leads one to believe that the nature of the assemblage of minerals and organic residues must play an important role in determining the sorption capacity of a soil for mercury vapor.

Of the total 500 μg of Hg^o vapor passed through the bell jar, 353 μg was adsorbed by soils, 111 μg was collected in the hopcalite traps, and 35 μg was unaccounted for (adsorbed on surfaces of the bell jar, petri dishes, tubing, etc.). The average Hg^o vapor uptakes were 8.48 ± 0.82 μg, 18.57 ± 0.62 μg, 18.06 ± 1.51 μg, 36.54 ± 2.74 μg and 6.57 ± 0.68 μg for the Arvada, Campspass, Heldt, Bainville and Terry soils, respectively. The concentration of Hg^o vapor was reduced from 75.5 μg/m^3 at the inlet, to 16.8 μg/m^3 at the outlet. Three factors would influence the removal of Hg^o vapor from the air, namely: 1) the sorbing power of soil, 2) the surface area, and 3) the duration of contact. Since the volume of the bell jar was 23 liters and the air flow rate was 100 ml/min, a complete air change in the bell jar required 230 minutes in which time 78% of the Hg vapor was sorbed. If the rate of sorption had remained constant, then 99% of mercury vapor could have been in a 12-hour period, given a stagnant situation.

Mercury Vapor Concentration

Five Montana soils and three clay minerals were exposed for 24 hours to air containing from 85.16 to 208.67 μg Hg^o/m^3 of air (Table 22.12). The sorption of mercury vapor increased with increasing mercury vapor concentrations.

TABLE 22.11. 24 HOUR SORPTION OF ^{203}Hg-ELEMENTAL VAPOR BY SOILS, CLAY MINERALS AND OTHER MATERIALS IN AN ATMOSPHERE CONTAINING 75.9 μg $^{203}Hg^{o}/m^{3}$

Soils, Minerals and Others	^{203}Hg Vapor Sorbed, μg
Arvada	0.018
Campspass	0.077
Heldt	0.076
Bainville	0.072
Terry	0.015
Sand	0.002
Kaolinite**	0.004
Bentonite**	0.021
Illite #35*	0.308
Montmorillonite #25*	0.008
Metabentonite #38*	0.059
Cellulose powder	0.004
Dry straw	0.011
Humic acid, technical	0.170
Peat**	0.148
Charcoal	2.943

* Purchased from Ward's Natural Science Establishment, Inc.

** Kindly provided by the Soils Department, Oregon State University.

This sorption may be expressed by Freundlich's adsorption equation:

$$\frac{x}{m} = kc^{n}$$

where x is the amount of mercury vapor sorbed in μg, C is the concentration of mercury vapor in μg Hg/m^3, m is the mass of adsorbent, n and k are constants. Expressed logarithmically, the equation takes the form:

$$\log \frac{x}{m} = \log k + n \log C.$$

By plotting $\log \frac{x}{m}$ against log C, a straight line was observed (Figure 22.16). The empirical k and n values are estimated by the least squares method from the experimental points. A higher k value is an indication of greater sorption.

Exposure Time

To determine sorption capacities, the minerals and soils were exposed to air containing an average Hg^{o} concentration of 79.2 μg/m^3 for 16-17 days. Sorption measurements were made daily initially and every other day later during the exposure period by temporarily removing each sample for a 10 minutes radioassay. The sorption of mercury vapor, although decreasing progressively with time, never reached a saturation state after 17 days of exposure (Figure 22.17). The average daily sorptions and ranges were 0.017 (.012-.025), 0.059 (.039-.077), 0.030 (.015-.080), 0.047 (.018-.072), and 0.010 (.006-.015) μg Hg per 2 g of the Arvada, Campspass, Heldt, Bainville, and Terry soils, respectively. These values were slightly less than the single day values reported in Table 22.11. The average Hg sorption by montmorillonite #25 was even lower than those of Arvada and Terry, and there was very little increase of uptake after 12 days. Illite had the highest rate of Hg sorption; the Hg sorptions of illite and metabentonite per unit time also decreased progressively with the time of exposure.

Nature of Mercury in Soil After Sorption

There was no loss of radioactivity in these soils after: 1) being placed in a vacuum dessicator for 24 hours, 2) being heated in an oven at 110°C for 2 hours, or 3) bubbling a stream of air through the suspension to remove the mercury vapor (Clarkson and Greenwood, 1970). Failure to remove any radioactivity by these treatments suggested that the sorbed mercury vapor must be either very tightly bound or transformed into a non-volatile form. Samples were analyzed by the method of Clarkson and Greenwood (1970), and only a small fraction of the sorbed mercury vapor was shown to have been converted to the mercuric form (Table 22.13). Other mercury forms remained unidentified.

Plant Uptake

Fifty seeds were sown and germinated in petri dishes containing soils exposed to mercury vapor as described above. Beacuse of extremely low radioactivity in the seeds and leaves, the counting error remained high even though samples were counted for 60 minutes. Conclusions from the presented data are therefore limited. It is apparent, however, that the mercuric mercury content of all soils was low (Tables 22.13 and 22.14) and was not necessarily a simple function of total mercury content. When the data were pooled for all sampling dates, there was a linear relationship ($P = 0.05$) between soil mercuric mercury content and the mercury content of seeds and leaves (Figure 22.18). There was no such relationship with total soil mercury.

CONCLUSION

Soils of different characteristics, clay minerals and various organic materials exhibited various degrees of sorption for mercury vapor. Among the clay minerals, illite had the highest sorption capacity, while kaolinite had the lowest. Different types of organic materials were also shown to have a wide range of sorption capacity; the highest being for humic acid and lowest for cellulose powder. Both the organic matter and mineralogical make-up of the soil may play an important role for the wide range of mercury vapor sorptions observed. The sorption phenomenon for soils was adequately described by the Freundlich type equation. The data indicated that the sorption of mercury vapor did not reach its maxima when the mercury vapor concentration was increased to 209 $\mu g/m^3$ or the soils were continually exposed for 17 days. Only a small fraction of mercury sorbed was transformed to mercuric form. The major portion of the mercury sorbed by soils remained unidentified and a further study of transformation is needed.

For studies into the uptake of mercury vapor by plants, a simple theory and plant chamber were employed to estimate total leaf resistance of whole plants to water vapor exchange. The estimates were independent of leaf temperature. The approach involved the measurement, at steady state conditions, of the net change in water vapor flux per unit leaf surface (Δq_v) in response to a small change in absolute humidity (ΔC_A). Assuming that total leaf resistance (r_L) was constant and that change in leaf temperature (T_L) was negligible, total leaf resistance was calculated from the following equation

$$r_L = \Delta C_A / \Delta q_v \ (\text{sec cm}^{-1})$$

Evidence is presented which indicates that such assumptions did not significantly alter estimates of r_L from the true values for changes in ambient relative humidity ranging from 0.011 to 0.074. Total leaf resistance of whole-plant communities estimated in this manner did not differ for ambient temperatures of 17, 25 and 35 C. Mean values of r_L ranged from 83 sec cm^{-1} in darkness to 2.4 sec cm^{-1} at an illumination of 12.8 klux.

Using a whole-plant chamber and ^{203}mercury, a quantitative study was made of the effect of environmental parameters on the uptake of metallic mercury vapor by wheat. Factors were examined in relation to their influence on components of the gas-assimilation model.

Reproduced from best available copy.

TABLE 22.14. UPTAKE OF ^{203}Hg BY GERMINATING WHEAT SEEDS FROM SOILS AFTER EXPOSURE TO ^{203}Hg-ELEMENTAL MERCURY VAPOR

			10 Seedlings		Leaves From 10 Plants	
	Total ^{203}Hg	Mercuric ^{203}Hg	2 days	3 days	7 days	10 days
Soils	µg	µg	ng	ng	ng	ng
Arvada	8.25	1.66	8.4	9.1	5.7	2.6
Campspass	20.03	0.42	6.5	9.7	2.3	1.5
Heldt	19.80	4.44	15.0	14.6	9.8	5.1
Bainville	40.05	4.04	15.0	18.1	4.6	6.2
Terry	7.20	1.97	7.6	6.5	2.6	1.6

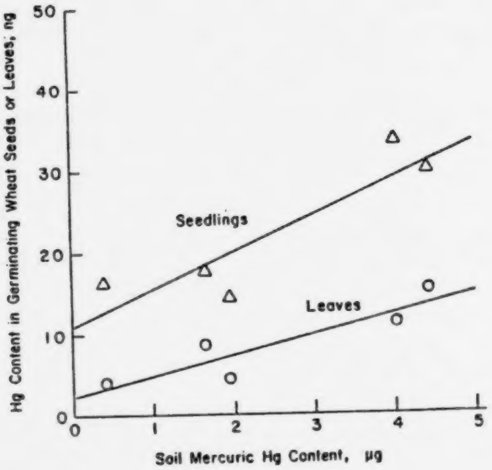


Figure 22.18. Plant uptake of ^{203}Hg from soils previously exposed to metallic ^{203}mercury vapor. Mercuric-Hg content of the dry soil expressed as µg Hg per 200 g soil. ^{203}Mercury content of wheat tissue expressed as ng Hg per 20 seedlings or leaves from 20 plants.

$$U(Hg) = \frac{C'_A - C'_L}{r_{L.Hg} + r_{M.Hg}}$$

where U(Hg) is the rate of mercury uptake per unit leaf surface, C'_A is the ambient mercury vapor concentration, C'_L is the mercury concentration at immobilization sites within the plant (assumed to be zero , $r_{L.Hg}$ is the total leaf resistance to mercury vapor exchange, and $r_{M.Hg}$ is the residual term to account for unexplained physical and biochemical resistances to mercury vapor uptake. $r_{L.Hg}$ was particularly influenced by illumination (o to 12.8 klux), but unaffected by ambient temperature (17 to 33 C) and mercury vapor concentration (0 to 40 $\mu g\ m^{-3}$. The principal limitation to mercury vapor uptake was $r_{M.Hg}$, which was linearly related to temperature, but unaffected by mercury vapor concentration and illumination, except for apparent high values in darkness. Knowing C'_A and estimating $r_{L.Hg}$ and $r_{M.Hg}$ from experimental data, mercury vapor uptake by wheat in light was accurately predicted for several durations of exposure using the above model.

Uptake of mercury vapor was found to differ between plant species, but the most pronounced difference was that which existed between plants possessing C_3 and C_4 photosynthetic pathways. In terms of the model employed, such differences were largely attributable to differences in the internal biochemical and physical resistances encompassed by the term $r_{M.Hg}$.

In vitro experiments with homogenized leaves revealed that the uptake and conversion of elemental mercury vapor to mercuric mercury by the leaves of various plant species was an enzymatic process. This process was in part light dependent. A non-specific oxidative enzyme such as catalase may also be involved, since mercury conversion was impaired by amitrole.

REFERENCES

Aston, M. J. 1976. Variation of Stomatal Diffusive Resistance with Ambient Humidity in Sunflower (*Helianthus annuus*). Aust. J. Plant Physiol., 3:489-501.

Bierhuizen, J. F., and R. O. Slatyer. 1964. Photosynthesis of Cotton Leaves Under a Range of Environmental Conditions in Relation to Internal and External Diffusive Resistance. Aust. J. Biol. Sci., 17:348-359.

Bradeen, D. A., and G. D. Winget. 1974. Site-specific Inhibition of Photophosphorylation in Isolated Spinach Chloroplasts by $HgCl_2$. II. Evidence for Three Sites of Energy Conservation Associated with Noncyclic Electron Transport. Biochim. Biophys. Acta, 333:331-342.

Campbell, G. S. 1975. Steady-state Diffusion Porometers. In: Measurement of Stomatal and Diffusive Resistance. Bull. 809; College of Agriculture Research Center, Washington State Univ.; pp. 20-23.

Clarkson, T. W., and M. R. Greenwood. 1970. Selective Determination of Inorganic Mercury in the Presence of Organomercurial Compounds in Biological Material. Anal. Biochem., 37:236-243.

Cowan, I. R., and F. L. Milthorpe. 1968. Plant Factors Influencing the Water Status of Plant Tissues. In: Water Deficits and Plant Growth, vol. 1, T. T. Kozlowski, ed. Academic Press, New York. pp. 137-193.

D'Itri, F.M. 1972. The Environmental Mercury Problem CRC Press, Cleveland Ohio.

Fujimura, Y. 1964. Studies on the Toxicity of Mercury. Jap. J. Hyg., 18:10.

Gates, D. M. 1968. Transpiration and Leaf Temperature. Ann. Rev. Plant Physiol., 19:211-238.

Hall, A. E., and M. R. Kaufmann. 1975. Stomatal Response to Environment with *Sesamum indicum* L. Plant Physiol., 55:455-459.

Horwitz, L. 1957. Observation on the Effect of Metallic Mercury upon Some Microorganisms. J. Cell. and Comp. Physiol., 49:437-454.

Hsiao, T. C. 1975. Variables Affecting Stomatal Opening--Complicating Effects. In: Measurement of Stomatal and Diffusive Resistance. Bull. No. 809; College of Agriculture Research Center, Washington State Univ.; pp. 28-31.

Jarvis, P. G. 1971. The Estimation of Resistances to Carbon Dioxide Transfer. In: Plant Photosynthetic Production. Manual of Methods. Z. Sestak, J. Catsky, and P. G. Jarvis, ed. Dr. W. Junk, N.V., Publishers. The Hague. pp. 566-631.

John, M. K. 1972. Mercury Uptake from Soil by Various Plant Species. Bull. Environ. Contam. Toxicol., 8:77-80.

Jost, W. 1952. Diffusion in Solids, Liquids and Gases. Academic Press, New York, 413 pp.

Mikhailov, V. K. and M. I. Kochegarova. 1967. Diffusion and Thermal Diffusion of Mercury Vapor in Air. In: Chem. Abstracts, 69:5777.

Milthorpe, F. L., and H. L. Penman. 1967. The Diffusive Conductivity of the Stomata of Wheat Leaves. J. Exp. Bot., 18:422-457.

Organization for Economic Cooperation and Development. Paris, 1974. Mercury Use and Emission. In: Mercury and the Environment. pp. 73-74.

Osman, A. M., and F. L. Milthorpe. 1971. Photosynthesis of Wheat Leaves in Relation to Age, Illuminance and Nutrient Supply: II. Results. Photosynthetica, 5:61-70.

Siegel, S. M., and B. Z. Siegel. 1975. Geothermal Hazards. Mercury Emission. Environ. Sci. Technol., 9:473-474.

Slatyer, R. O. 1967. Plant-water Relationships. Academic Press, New York. p. 14.

Slatyer, R. O. 1971. Effect of Errors in Measuring Leaf Temperature and Ambient Gas Concentration on Calculated Resistance to CO_2 and Water Vapor Exchange in Plant Leaves. Plant Physiol., 47:269-274.

Slatyer, R. O., and J. F. Bierhuizen. 1964. Transpiration from Cotton Leaves Under a Range of Environmental Conditions in Relation to Internal and External Diffusive Resistances. Aust. J. Biol. Sci., 17:115-130.

Spier, J. L. 1940. The Determination of the Coefficient of Diffusion of Mercury Vapor and Cadmium Vapor in Nitrogen. Physica, 7:381-384.

Tanner, C. B. 1972. Psychrometers in Micrometeorology. In: Psychrometry in Water Relations Research, R. W. Brown and B. P. Van Haveren, eds. Utah Agric. Exp. Sta., Utah State Univ., pp. 239-247.

Trost, P. B., and R. E. Bisque. 1972. Distribution of Mercury in Residual Soils. In: Environmental Mercury Contamination, R. Hartung and B. D. Dinman, eds. Ann Arbor Science Publisher Inc., Ann Arbor, Mich. pp. 178-196.

Van Haveren, B. P., and R. W. Brown. 1972. The Properties and Behavior of Water in the Soil-Plant-Atmosphere Continuum. In: Psychrometry in Water Relations Research, R. W. Brown and B. P. Van Haveren, ed. Utah Agric. Exp. Sta., Utah State Univ., pp. 1-27.

SECTION 23

EFFECTS OF STACK EMISSIONS UPON PRIMARY ASPECTS OF PHOTOSYNTHESIS AND PHOTOSYNTHETICALLY-LINKED NITROGEN FIXATION

R. M. Tetley and N. I. Bishop

ABSTRACT

The toxicity of selected trace elements emitted from coal-fired power plants was determined for several biological functions in the blue-green algae, *Anabaena cylindrica*. This organism performs the basic biological functions of nitrogen fixation, photosynthesis, respiration, and growth, was easily adaptable to existing measurement techniques, is of significance in the grassland biome, and provides an excellent multifunctional organism for testing the effects of emission contaminants. The elements tested were F, Na, Cl, Br, Li, K, Sr, Ba, Cr, Mn, Ni, Cu, Zn, Cd, Hg, Pb, and As. In order of decreasing toxicity Hg, Cu, Cr, Ni, Cd, and Pb exerted strong inhibition at levels of 1 mM or below. In assays of biological functions Hg, Cu, Zn, Cd, and Pb exhibited strong toxicity at 1 mM or lower levels. Considerably greater sensitivity was seen with Cr, Ni, Mn, Cu, Sr, and Hg in growth studies than in physiological assays. The metabolic inhibitors KCN, DCMU, *m*-Cl-CCP, and DBMIB, and the interaction of light and oxygen on nitrogenase activity were studied and compared to metal toxicity effects. These studies resulted in the recognition of methodology artifacts which should be acknowledged in field and laboratory nitrogen fixation studies. In the strain of *Anabaena* used, and under controlled conditions, hydrogen production serves as a sensitive measure of nitrogen fixation.

INTRODUCTION

In establishing a short-term program capable of surveying the biological impact of trace elements present in the stack emissions of coal fired power plants, it was important to choose an appropriate biological system. The criteria used to select an organism or system for this study were that it performed several basic biological functions, that it was easily adaptable to existing measurement and assay techniques, and that it was of significance in the grassland biome as well as having importance in other environmental settings. The decision to use blue-green algae for the organism in this study was made because they fit the previous criteria.

Blue-green algae have received increased attention recently and their biology is surveyed thoroughly in two relatively new reviews (Carr and Whitton, 1973; Fogg, *et al.*, 1973). Certain blue-green algae are capable of fixing atmospheric nitrogen gas into biologically usable ammonia as well as functioning in the role of photosynthetic primary producers. Therefore, apart from the photosynthetic bacteria, the growth and metabolism of the blue green algae is unique in that it includes the basic biological functions of nitrogen fixation, photosynthesis, respiration, and growth. As will be discussed in the methods portion of this section, it is possible to grow blue-green algae in a manner suitable to measure these biological functions with existing instrumentation. Although blue-green algae have a broad aquatic distribution, including lakes, streams, hot springs, estuaries, salt marshes, and marine habitats, their importance in soils is becoming more and more apparent. Blue-green algae are extremely abundant in certain soils and function to retard erosion, maintain moisture, add organic content, and fix nitrogen. The terrestrial algae are considered to be most abundant in rich moist soils, but may be the dominant microflora in arid soils.

Blue-green algae are also responsible for large inputs of nitrogen into certain tropical agricultural soils (MacRae and Castro, 1967). However, high fixation rates have also been observed in pasture and meadow land in temperate regions (Henriksson, 1971) as well in arid regions of the United States (Mayland *et al.*, 1966). In addition, it has been generally established that nitrogen fixed by soil blue-green algae eventually becomes available to other associated organisms such as higher plants.

The vertical distribution of blue-green algae in soil may extend to more than a meter, but because of their obligate photoautotrophic nature, they are most abundant near the soil surface. It is reasonable to assume that deposition of particulate matter containing trace quantities of various elements would have early effects on the microalgae present in this zone. It appears, therefore, that nitrogen fixing blue-green algae are not only superb multifunctional organisms for testing the effects of emission contaminants on biological function, but they are also important organisms which would be affected by such contaminants in the grassland biome.

For these reasons then, this study was undertaken to investigate the effects of trace elements on photosynthesis, nitrogen fixation, respiration and growth in the blue-green algae, *Anabaena cylindrica* and the water ferns *Salvinia* and *Azolla*.

In addition to the trace elements being tested, several metabolic inhibitors, whose mode of action is well known in other systems, were included to assist in interpreting the action of active trace elements. The role of oxygen in stimulating and inhibiting hydrogen production (nitrogen fixation) was also investigated and related to the inhibitor study. As a check of the sensitive hydrogen production assay used to determine nitrogenase activity, a series of experiments were conducted using the acetylene reduction assay for comparison.

MATERIALS AND METHODS

Culturing of blue-green algae

The blue-green algae chosen for assays of trace metal effects was *Anabaena cylindrica* Lemm. (B629). This organism has demonstrated an active nitrogenase activity which may be assayed by measuring hydrogen production (Jones and Bishop, 1976). Cells were grown on the medium described by Castenholz (1970) without the addition of nitrate. The nitrogen remaining in the iron chelator does not inhibit the production of the nitrogenase. Cells were grown at a density of approximately 3.5 µl packed cell volume (PCV) per ml of culture medium in a constant density growth apparatus similar in function and design to that described by Senger and Wolf (1964), but fitted with overflow and sampling ports on the side of the tube. Cultures were illuminated at a light intensity of about 8 x 10^4 ergs/cm^2/sec from mixed fluorescent tubes supplemented with tungsten bulbs. Culture temperature was maintained at 24 to 27 C and aerated with 2% CO_2 in air. Continuous cultures were renewed occasionally from batch grown stock cultures which were subcultured every four to six days.

Assay Procedures

Single samples of algae were prepared for assay by removing 10 to 12 ml aliquots from the growth tube and concentrating the cells by centrifugation. PCV values were determined by centrifuging 10 ml of the culture at 1250 x G for 5 minutes in Constable protein tubes. For analysis cells were resuspended in 1.8 to 2.0 ml of culture media and placed in small 10 to 25 ml Erlenmeyer flasks capped with serum stoppers. The gas over the cell suspensions was removed three times by aspiration and replaced by argon or helium after each evacuation. The cells were stored anaerobically in the absence of nitrogen for short periods before assay. Various gas mixtures were produced by adding measured volumes of gas with syringes to cell samples that had been evacuated an additional time. The balance of the pressure was provided with argon or helium. After addition of the cells to the cuvette, they were treated in the following manner (see Figure 23.1): 1) addition of 5% of the cuvette volume of test solution and dark incubation for 3 to 5 minutes, 2) irradiation with white light with an intensity of approximately 7.5 x 10^4 ergs/cm^2/sec for three to six minutes until oxygen and hydrogen production rates were established, and 3) a dark period of three to six minutes in which respiratory rates were established. If photosynthesis was inhibited during the light irradiation steps, oxygen was added to the cuvette to establish a respiratory rate at an oxygen concentration of about 100 µM.

Changes in oxygen and hydrogen concentrations were monitored by an apparatus similar to that described by Jones and Bishop (1976). Two Yellow Springs Instrument 4004 Clark type electrodes were fitted to a 1.30 ml jacketed plexiglass cuvette maintained at 27 C and mixed by a small magnetic stirrer. Polarization voltages of -0.8 and +0.6 volts were maintained on the oxygen and hydrogen platinum electrodes respectively. Electrode currents were amplified and scaled by two Keithley electrometers (Models 601 and 602) and recorded on a dual pen recorder (Linear Instruments Corp., Model 291). Illumination was provided by a Tiyoda (Model 7034) variable output lamp. Argon, helium, and hydrogen were obtained from Airco, Inc. and C. P. Grade ethylene gas used as an ethylene standard was supplied by Matheson Gas Products. All trace elements tested were of reagent grade and commonly available from chemical companies. Test solutions produced by serial dilution of concentrated stock solutions of trace elements were purged with argon or helium and stored oxygen and nitrogen free prior to their addition to the reaction cuvette with small syringes. Oxygen and hydrogen responses of the electrodes were calibrated with culture media in equilibrium with air for 20.9% oxygen and 2 or 10% hydrogen and the values used were 247.8, and 15.0 or 74.9 μmolar respectively at 27 C (Dean, 1973; Loomis, 1928). Gas production and utilization rates were calculated in the units that were used in the measurements, *i.e.* pico- or nanomoles per microliter PCV per minute. One μl PCV represents about 0.5 nmoles or μgrams of chlorophyll-a. Respiration, light-induced hydrogen production, and gross photosynthesis (oxygen production in the light plus oxygen uptake in the dark) rates were plotted against inhibitor concentration and curves were drawn through the points by inspection. The criteria for choosing the trace elements tested in this study were twofold: 1) those that were present in the stack emissions of coal fired power plants at rates above 15 mg per second (Montana State Department of Natural Resources and Conservation, 1974) and, 2) those that have demonstrated toxic effects in other biological systems. Several elements fitting the first criteria were not tested. The elements were provided as the soluble anions or cations of either sodium or chloride salts, or as the acetate salt in the case of lead. Chromium and arsenic were supplied as $NaCr_2O_7$ and Na_2HAsO_4, respectively.

Gas chromatography

Experiments involving the acetylene reduction assay for nitrogenase activity were carried out by simultaneously running all samples in one experiment. Cells were concentrated by centrifugation and resuspended in culture media to a density of 7.5 to 15 μl per ml. Aliquots of 5 ml were pipetted to 25 ml flasks and capped with serum stoppers. The cells were rendered oxygen- and nitrogen-free as in the polarographic assays, but with the addition of 10% (4.02 *mM* in solution) acetylene generated from cold water and reagent grade calcium carbide. Reaction mixtures were incubated for 5 to 50 minutes on a modified Gilson respirometer shaking bath held at 27 C and supplied with tungsten light providing an intensity of 7.5×10^4 ergs/cm^2/second. After the desired reaction time, 0.5 ml samples of the gas mixtures over the cells were taken with 1.0 ml plastic tuberculin syringes. Assay of the ethylene present was accomplished on a Hewlett Packard Gas Chromatograph (Model 5830 A) with flame ionization detection. An Alltech aluminum column (6 ft. x 1/8 in. O.D.) packed with 80-100 mesh Porapak R was used with an oven temperature of 45 C and a flow rate of approximately 30 ml per minute. Areas under the peaks were standardized using 1% ethylene gas in air.

Growth studies

Anabaena cylindrica

The long term effect of trace metals on algal growth was determined by inoculating culture media containing a series of concentrations of the element under study with *Anabaena cylindrica*. Cells were grown for 48 to 56 hours in 18 x 150 mm Pyrex culture tubes, illuminated with fluorescent lights at an intensity of 2×10^4 ergs/cm^2/sec, and were bubbled with 2% carbon dioxide in air. After the growth period the total chlorophyll-a content of each tube was extracted with 10 ml of 80% acetone for at least 24 hours in the dark at room temperature. The chlorophyll-a was quantified by measuring the absorbance at 665 nm and employing the extinction coefficient given by Vernon (1960). The increase in chlorophyll content was determined by subtracting the initial value. The data are plotted as percent increase in chlorophyll of the untreated growth control. Cell clumping in single tubes occasionally resulted in high chlorophyll values.

The compounds used to study the effect of trace elements on the selected vital functions of *Anabaena cylindrica* were NaF, NaCl, NaBr, LiCl, KCl, $SrCl_2$, $BaCl_2$, $Na_2Cr_2O_7$, $MnCl_2$, $NiCl_2$, $CuCl_2$, $ZnCl_2$, $CdCl_2$, $HgCl_2$, Pb(acetate)$_2$, and Na_2HAsO_4.

Salvinia and *Azolla*

Samples of healthy *Salvinia* or *Azolla* fronds grown in departmental greenhouses were surface sterilized with sodium hypochlorite, and washed thoroughly with water. Uniform sized plants were inoculated into 50 ml volumes of culture media containing trace elements in 125 ml Erlenmeyer flasks. The water ferns were grown under fluorescent lights in growth chambers with a 16 and 8 hour day-night light regime. Visual and gravimetric observations were made of the growth over a period of several weeks in which time trace element toxicity could be determined.

The elements tested were Hg, Cr, Pb, Zn, Cd and Sr, between the concentrations of 0.001 and 1 *m*M.

RESULTS AND DISCUSSION

The Action of Oxygen and Select Metabolic Inhibitors on Photosynthesis, Respiration and Nitrogen Fixation

Oxygen and Nitrogenase Activity

A sample experimental trace (Figure 23.1), taken from the control data of the $ZnCl_2$ assays (Figure 23.19) illustrates a commonly observed phenomenon. After the light stimulated oxygen and hydrogen evolution rates had been

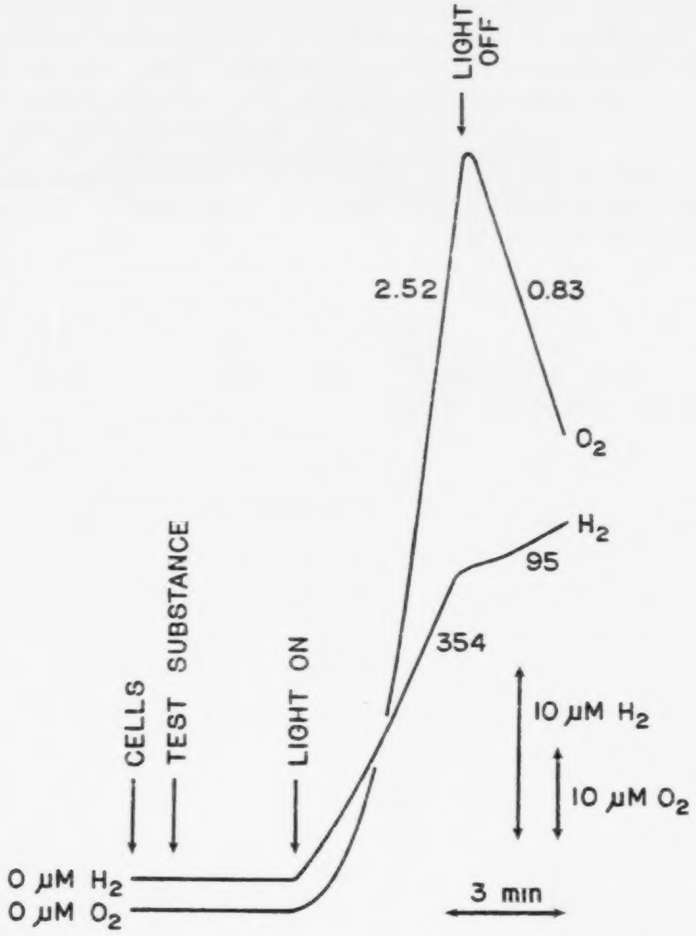


Figure 23.1 Sample tracing for the determination of respiration, photosynthesis, and hydrogen evolution in physiological assays of *Anabaena cylindrica*. Slope values are given as nmoles/µl PCV/min for oxygen, and pmoles/µl PCV/min for hydrogen.

established, the oxygen levels in the cuvette were of considerable magnitude (greater than 50 μM). Due to the oxygen accumulation during this first portion of the experimental run, a continued evolution of hydrogen was observed in the dark. The initial oxygen concentration of these experiments was purposefully brought to zero to minimize any possible deleterious effect on the sensitive nitrogenase (Drozd and Postgate, 1970; Haaker and Veeger, 1977). However, it is apparent that oxygen supports the nitrogenase activity in the dark. In order to determine if the assays performed were optimal for nitrogenase activity, a series of experiments were conducted, the results of which are summarized in Figures 23.2 and 23.3. It had previously been determined (Jones and Bishop, 1976) that the light-driven nitrogenase activity was well saturated at an intensity of 10^5 ergs/cm^2/second. However, oxygen addition in the dark may account for as much as 40% of the hydrogen production activity seen in saturating light (Tetley and Bishop, 1977). Figure 23.2 indicates that the hydrogenase activity due to light and that due to oxygen are partially additive at light intensities below about 7.5×10^4 ergs/cm^2/sec, and at saturating light intensities moderate oxygen concentrations have little or no effect. However, at high oxygen concentrations, in fact those

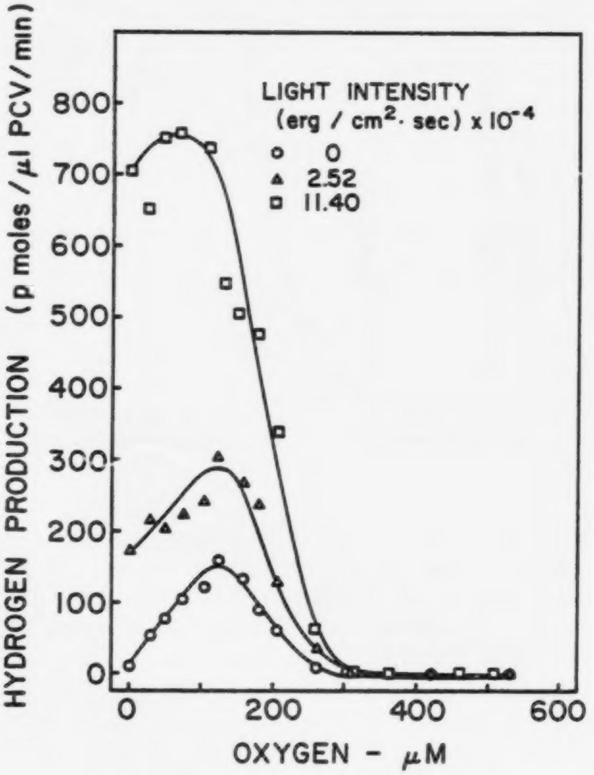


Figure 23.2 The effect of oxygen concentration and light intensity on hydrogen production in *Anabaena cylindrica*.

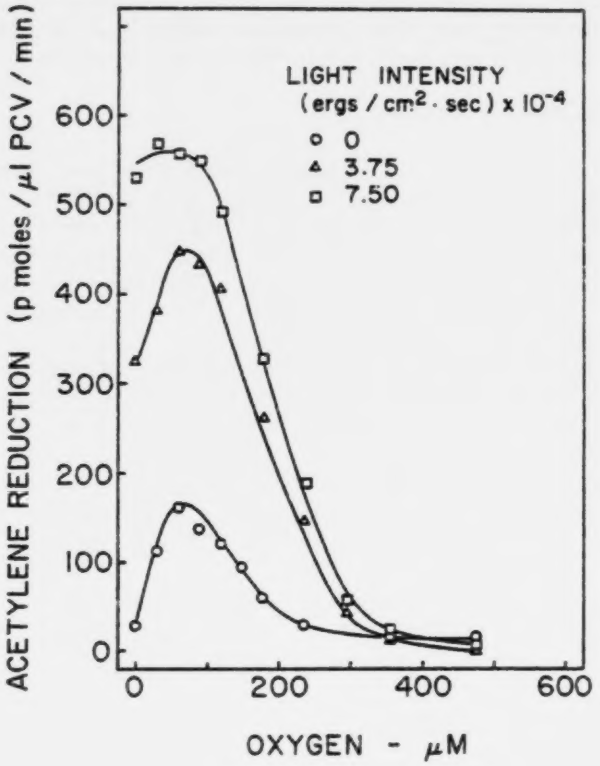


Figure 23.3 The effect of oxygen concentration and light intensity on acetylene reduction in *Anabaena cylindrica*.

at which the algae were grown, strong inhibition of the nitrogenase activity was seen; but clearly this was not an oxygen inactivation of the enzyme. At higher oxygen concentrations (*i.e.* 250 µM and above) an irreversible inhibition of the nitrogenase is found. It was observed in a similar series of experiments (Figure 23.3) that the same interaction between light and oxygen are observed when analyzing the nitrogenase activity with the standard acetylene reduction assay. From these experiments it is concluded that maximal hydrogen production is a good indication of acetylene reduction or nitrogen fixation, and may be obtained in saturating light at oxygen concentrations below about 100 µM. Normally the hydrogen evolution showed somewhat lower values than the acetylene reduction assays though there was considerable variability from experiment to experiment in both assays on the basis of packed cell volume. An interesting point was illustrated by the light and oxygen concentration study that bears directly upon measurement of actual nitrogen fixation rates during culture growth or natural growth in the field, as opposed to maximal nitrogenase activity. If the cells are not assayed under exactly the same conditions that they grow naturally, with respect to light intensity and gas concentrations, then grossly erroneous estimations of field or culture nitrogen fixation rates may result.

Effects of Select Metabolic Inhibitors

The compounds employed as model metabolic inhibitors were potassium cyanide, carbonyl cyanide *m*-chlorophenyl-hydrazone (*m*-Cl-CCP), 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), and 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB). Cyanide is effective in inhibiting oxygen uptake at the site of cytochrome oxidase in respiring organisms and inhibits electron transport at the site of plastocyanin in photosynthetic systems (Webster and Frenkel, 1953; Ouitrakul and Izawa, 1973). At millimolar levels, cyanide acts as a substrate for nitrogenase (Rivera-Ortiz and Burris, 1975) and therefore it has several potential sites of action in *Anabaena*. The action of cyanide on photosynthesis, respiration, and hydrogen production is illustrated in Figure 23.4. It is clear that 30 µM cyanide maximally inhibits respiration about 50% at about 100 µM O_2 levels, and completely eliminates oxygen-dependent hydrogen evolution in the dark. Light-stimulated hydrogen production is not inhibited to the same extent as the dark process.

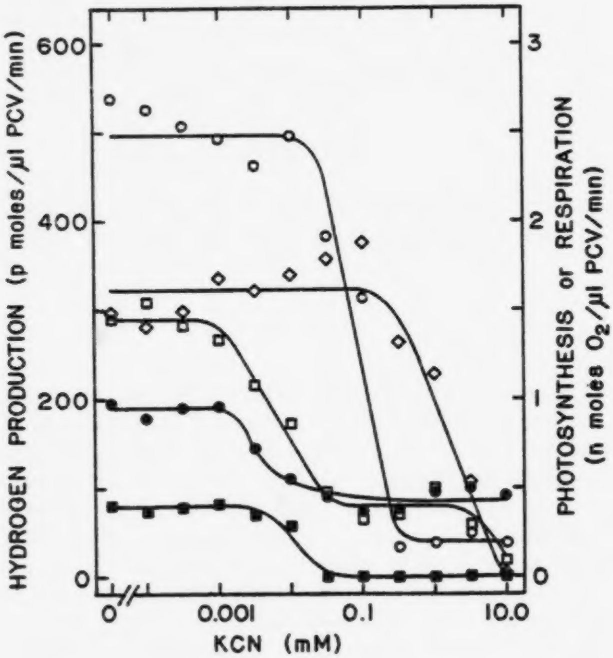


Figure 23.4. The effect of cyanide on respiration (●), photosynthesis (○), oxygen-stimulated hydrogen production in the dark (■), light-driven hydrogen production (□), and light-driven hydrogen production in the presence of DCMU (◇) in *Anabaena cylindrica*.

In fact, in the presence of DCMU, which prevents the evolution of oxygen (Figure 23.6), the inhibition of light-driven hydrogen evolution is seen at much higher cyanide concentrations. These experiments indicate that it is the oxygen produced during photosynthesis that is detrimental to light-driven hydrogen evolution at those cyanide concentrations sufficient to inhibit respiration. It appears that cyanide prevents the utilization of oxygen near the site of the nitrogenase, which in turn results in an oxygen-inactivation of this oxygen sensitive enzyme complex. Therefore, by inhibiting oxygen uptake, the nitrogenase may be affected indirectly by increased oxygen concentrations and the results could be easily misinterpreted as a direct effect. This is especially true in nitrogenase assays involving longer periods of time, such as the acetylene reduction assay. The inhibition of photosynthetic oxygen production at higher concentrations of cyanide is clearly a second effect supposedly due to electron transport inhibition at the level of plastocyanin. In DCMU-inhibited cells, cyanide does not exert a noticeable inhibition on light-stimulated hydrogen production until 300 μM levels are reached (Figure 23.4), whereas inhibition of photosynthesis, as judged by gross oxygen production, is affected at 30 μM KCN. If the inhibition at the plastocyanin level is the cause of the decrease in oxygen production it would not be expected that hydrogen production would continue at nearly control rates. This is particularly noticeable at 300 μM cyanide where photosynthesis is inhibited by over 90%. For these reasons, it is possible that cyanide inhibits photosynthesis in photosystem II. Alternatively, photosynthesis in heterocysts may be protected from cyanide by competing for cyanide-binding sites, for cyanide clearly rapidly enters the heterocysts as evidenced by its effect on oxygen-stimulated hydrogen production in the dark.

Carbonyl cyanide *m*-chlorophenyl hydrazone or *m*-Cl-CCP is a potent poison which uncouples oxidative and photosynthetic electron transport from phosphorylation (Heytler and Pritchard, 1962; Biggins, 1969). Since the nitrogenase is dependent upon ATP produced by these processes, uncoupling should result in a loss of nitrogenase activity. From Figure 23.5 it is clear that hydrogen production in both the dark and the light is eliminated by 3 to 10 μM *m*-Cl-CCP. However, photosynthesis is retarded about 60% and respiration slightly increased at the same level of inhibitor. Normally uncouplers cause increases of 50% or more in respiration. In the case of photosynthetic electron transport (oxygen production) this compound also shows strong inhibition. Thus the inhibitory effect of light-driven hydrogen production may be attributed to uncoupling, since electron transport is reported to be inhibited in photosystem II by *m*-Cl-CCP (Kimimura, *et al.*, 1971). DCMU inhibition of photosynthetic electron transport has been well documented (Bishop, 1958). Figure 23.6 shows no effect of DCMU on either respiration or light-stimulated hydrogen production. However, complete inhibition of oxygen production is observed at about 1 μM levels. Since gross photosynthesis is calculated as the difference in oxygen production rate in the light and uptake in the dark in the presence of oxygen, there is apparent photosynthesis at DCMU levels above 0.5 μM. This is interpreted as an inhibition of respiration. Since no oxygen is produced in the presence of DCMU, then complete inhibition of gross photosynthesis is seen when the photosynthetic activity is assayed in the absence of oxygen. Light inhibition of respiration has been recognized in blue-green algae for some time

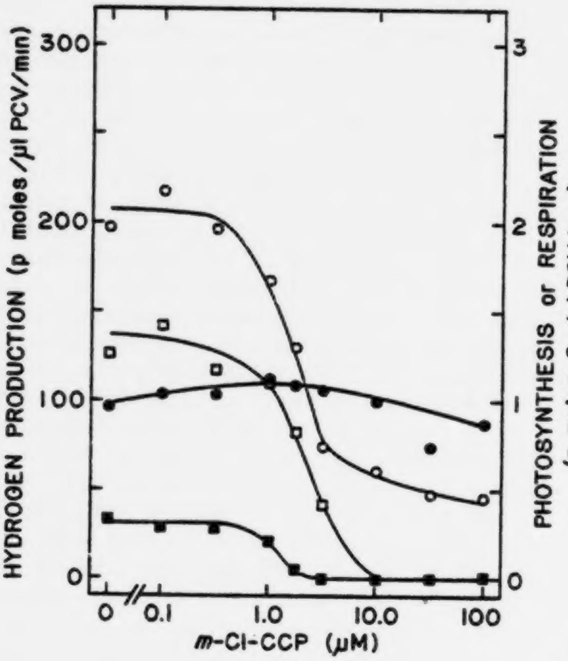


Figure 23.5. The effect of *m*-Cl-CCP on respiration (●), photosynthesis (○), oxygen-stimulated hydrogen production in the dark (■), and light-driven hydrogen production (□) in *Anabaena cylindrica*.

(Brown and Webster, 1953). The light inhibition of respiration decreases with increases in oxygen concentration and appears to involve the cyanide-sensitive component of oxygen uptake. Therefore, between experimental runs it is important to measure both respiration and photosynthetic activities at the same oxygen concentration. Though DCMU is effective in blocking non-cyclic electron transport, photosystem I driven cyclic photophosphorylation apparently continues, and sufficient ATP synthesis is maintained, resulting in no loss of ATP-dependent hydrogen evolution.

The action of DBMIB in photosynthesis has been recognized more recently (Trebst *et al.*, 1970). This compound acts at the site of plastiquinone, and is a strong inhibitor of oxygen production as well as photosynthetic phosphorylation. The action of DBMIB on *Anabaena* is seen in Figure 23.7. Oxygen production is inhibited about 60% at 10 µM DBMIB, and maximally inhibited at about 30 µM. A parallel inhibition of light-induced hydrogen production is also seen and is complete. At 10 µM levels of the inhibitor, oxygen stimulation of hydrogen production in the dark is not inhibited

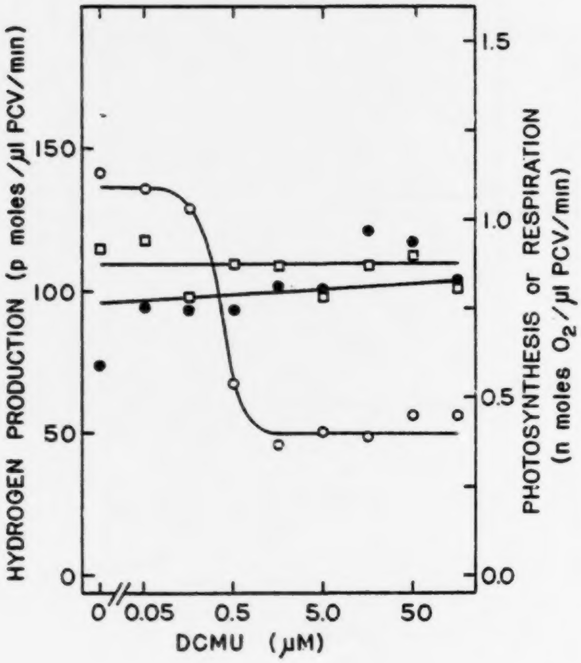


Figure 23.6. The effect of DCMU on respiration (●), photosynthesis (○), and light-driven hydrogen production (□) in *Anabaena cylindrica*.

though higher concentrations of DBMIB are effective. Oxygen uptake is unaffected by DBMIB up to 100 µM levels. Apparently DBMIB acts as a selective inhibitor of the light-stimulated nitrogenase activity before influencing the aerobic dark production of hydrogen at higher concentrations. This information, taken together with the selective cyanide inhibition data of aerobic dark hydrogen production indicates that the light and the dark ATP generating systems necessary for driving the nitrogenase operate independently of one another.

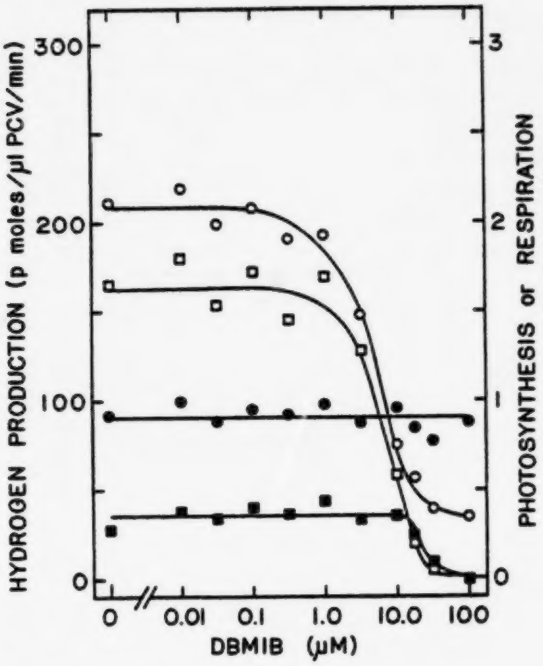


Figure 23.7. The effect of DBMIB on respiration (●), photosynthesis (○), oxygen-stimulated hydrogen production in the dark (■), and light-driven hydrogen production (□) in *Anabaena cylindrica*.

Effects of Trace Elements on Photosynthesis, Nitrogen Fixation, Respiration and Growth in *Anabaena*

The effect of investigated trace elements upon respiration, photosynthesis, light-driven hydrogen production and growth in *Anabaena cylindrica* is shown in Figures 23.8 to 23.23. The composite results of photosynthesis, nitrogen fixation, respiration and growth are tabulated in Figures 23.24 through 23.27, respectively.

Of the 17 trace metal contaminants tested, five may be singled out as producing generally toxic effects in short-term assays of *Anabaena* function. These are Hg, Cu, Zn, Cd, and Pb in the order of their approximate decreasing toxicity to photosynthesis, nitrogen fixation, or respiration. It would be expected that any one of these processes would be limiting to growth, and therefore the process most sensitive to a toxic element would determine the tolerance of the organism to that element. As anticipated, in no instance

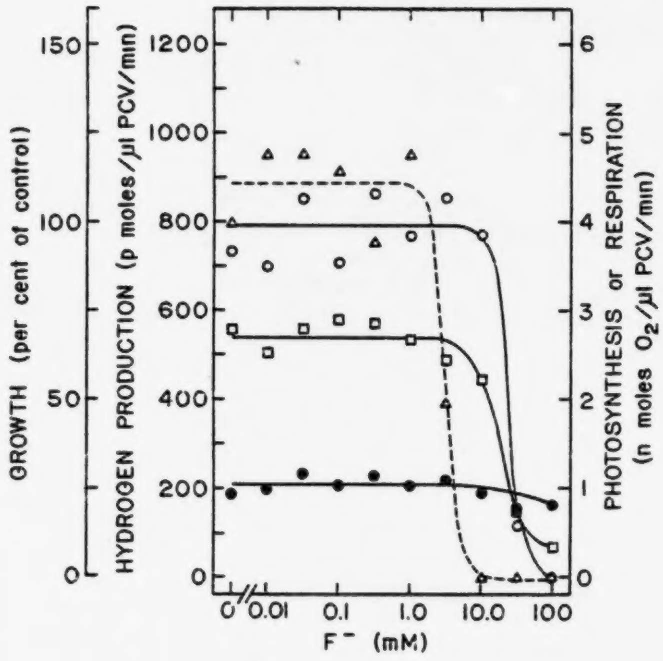


Figure 23.8. The effect of F as NaF on respiration (●), photosynthesis (○), light-driven hydrogen production (□), and growth (△) in *Anabaena cylindrica*.

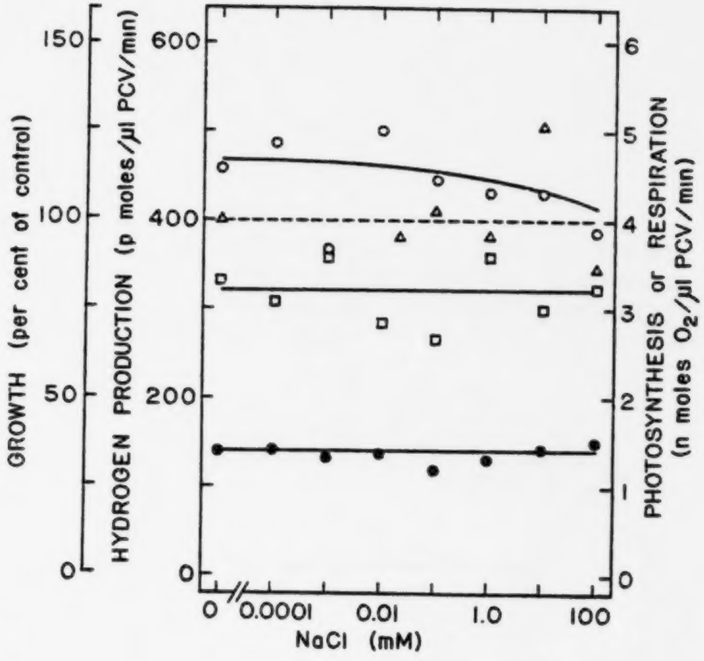


Figure 23.9. The effect of Na and Cl as NaCl on respiration (●), photosynthesis (○), light-driven hydrogen production (□), and growth (△) in *Anabaena cylindrica*.

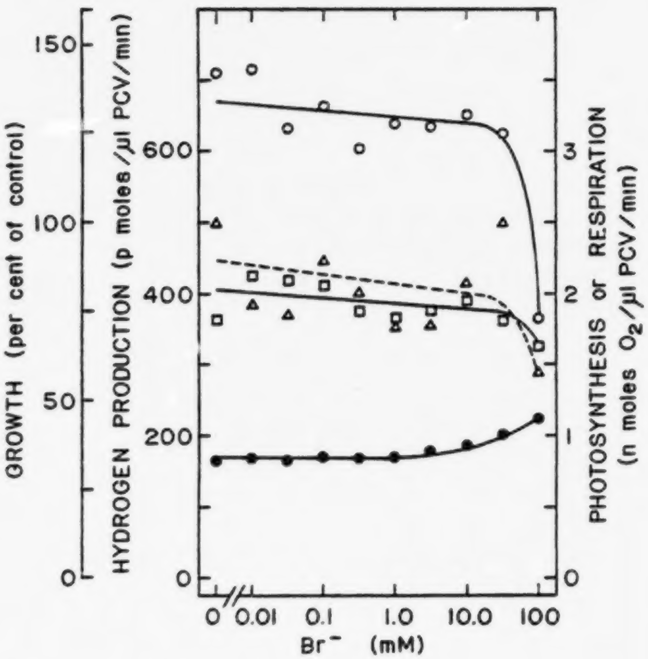


Figure 23.10. The effect of Br as NaBr on respiration (●), photosynthesis (○), light-driven hydrogen production (□), and growth (△) in *Anabaena cylindrica*.

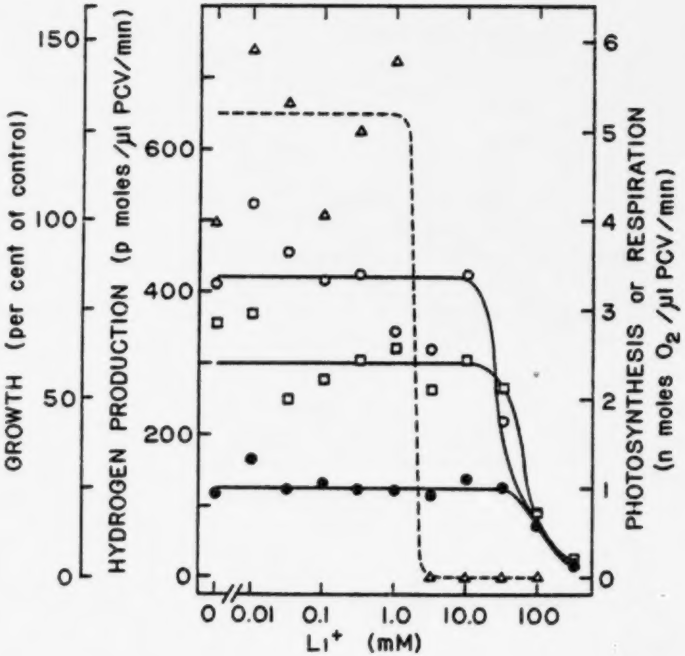


Figure 23.11. The effect of Li as LiCl on respiration (●), photosynthesis (○), light-driven hydrogen production (□), and growth (△) in *Anabaena cylindrica*.

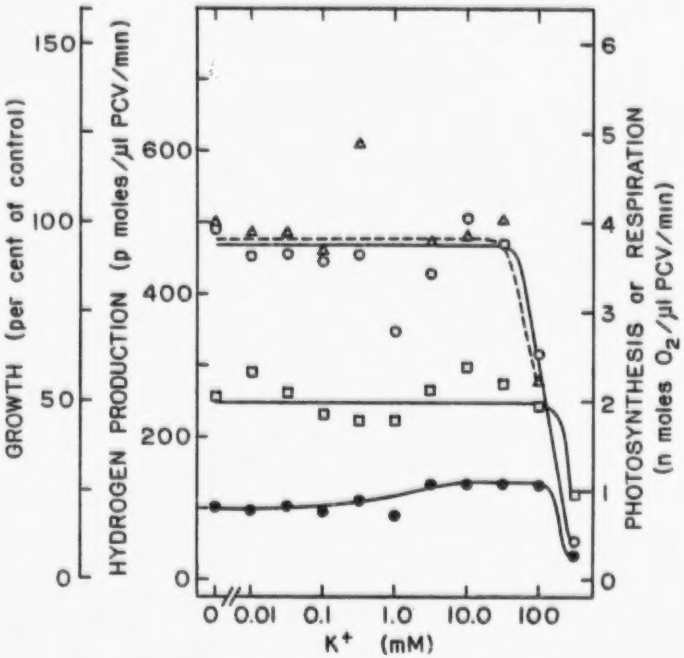


Figure 23.12. The effect of K as KCl on respiration (●), photosynthesis (○), light-driven hydrogen production (□), and growth (△) in *Anabaena cylindrica*.

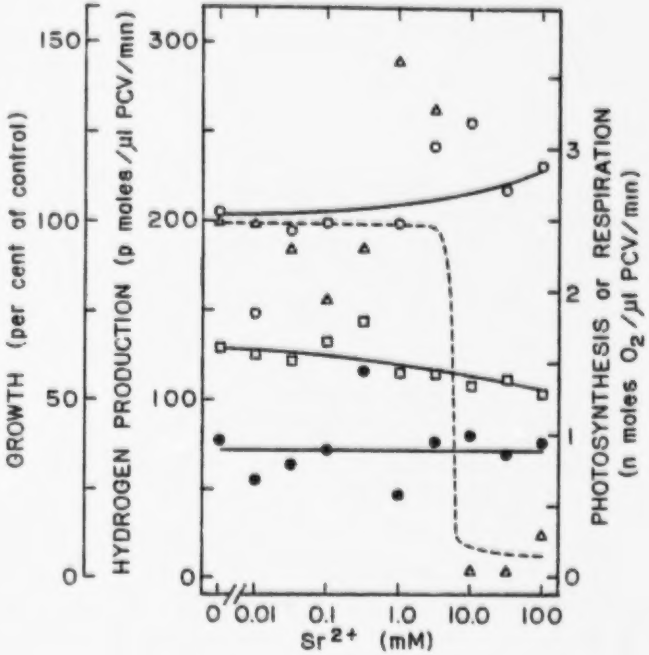


Figure 23.13. The effect of Sr as $SrCl_2$ on respiration (●), photosynthesis (○), light-driven hydrogen production (□), and growth (△) in *Anabaena cylindrica*.

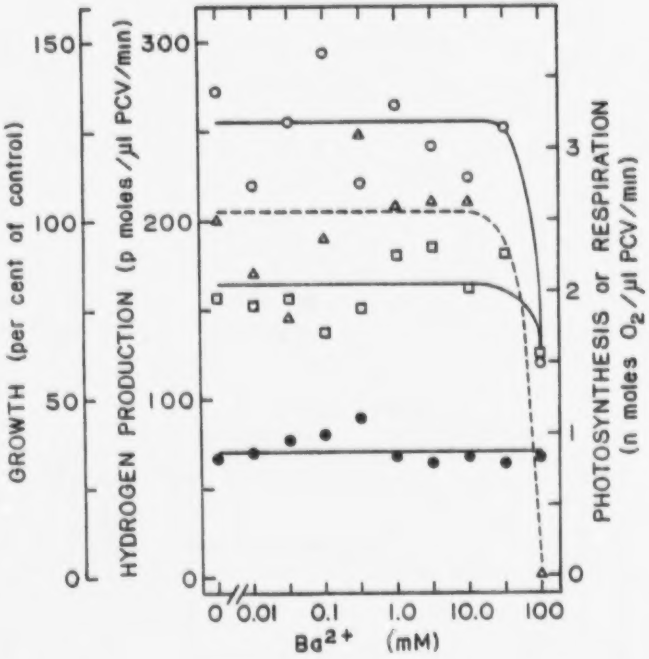


Figure 23.14. The effect of Ba as $BaCl_2$ on respiration (●), photosynthesis (○), light-driven hydrogen production (□), and growth (△) in *Anabaena cylindrica*.

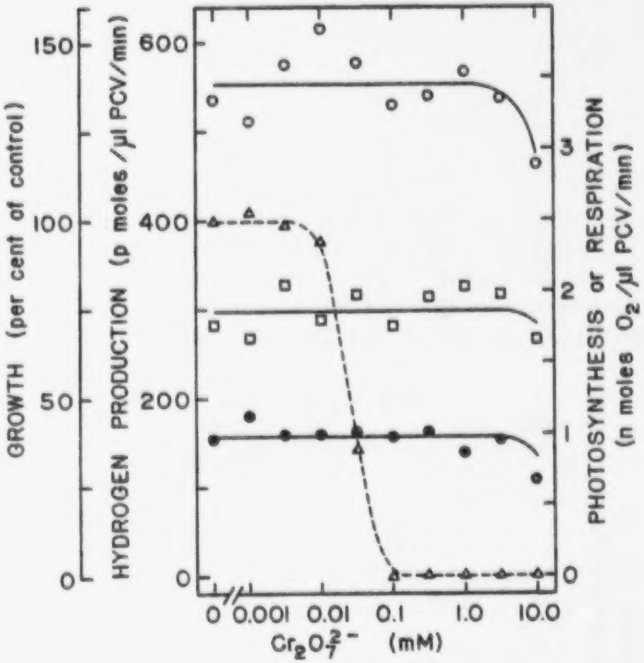


Figure 23.15. The effect of Cr as $Na_2Cr_2O_7$ on respiration (●), photosynthesis (○), light-driven hydrogen production (□), and growth (△) in *Anabaena cylindrica*.

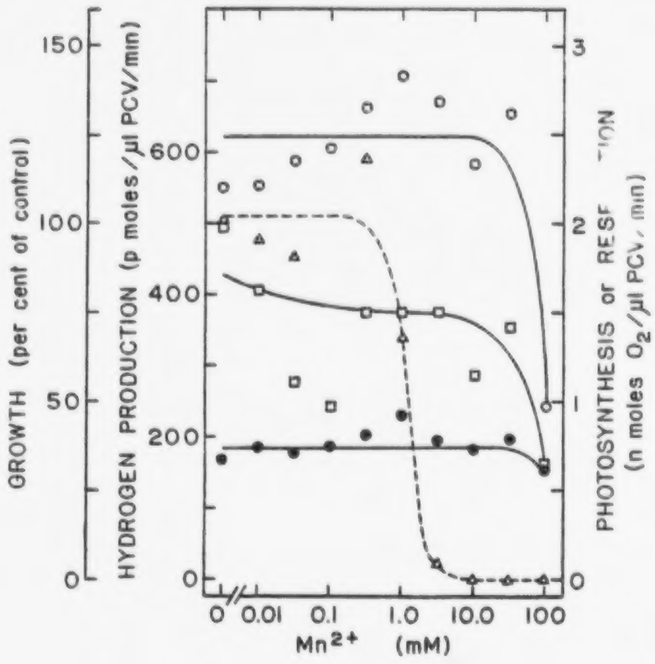


Figure 23.16. The effect of Mn as $MnCl_2$ on respiration (●), photosynthesis (○), light-driven hydrogen production (□), and growth (△) in *Anabaena cylindrica*.

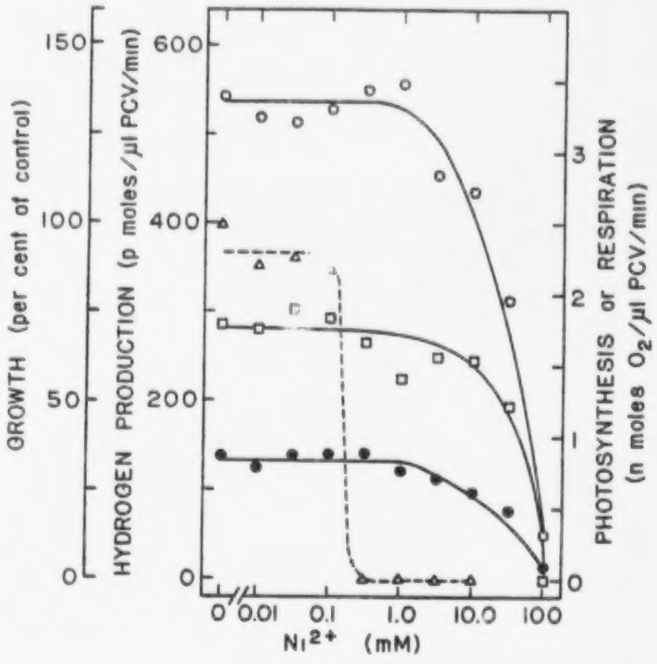


Figure 23.17. The effect of Ni as $NiCl_2$ on respiration (●), photosynthesis (○), light-driven hydrogen production (□), and growth (△) in *Anabaena cylindrica*.

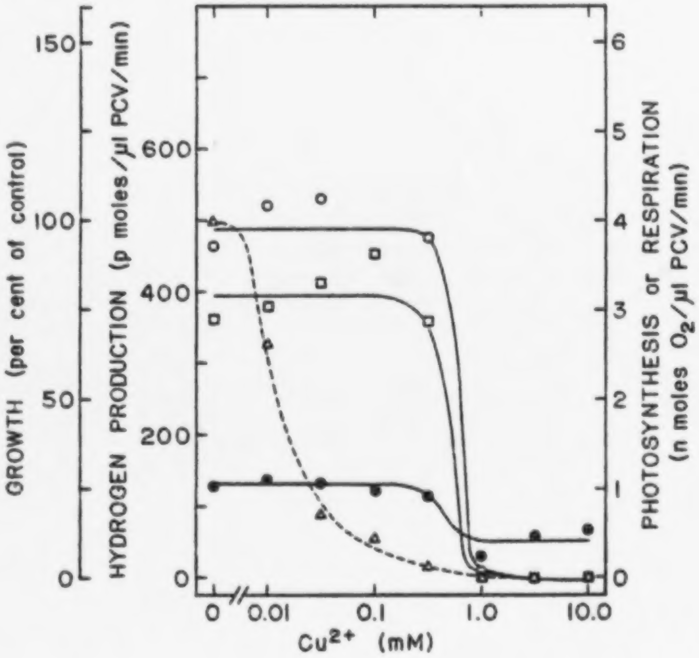


Figure 23.18. The effect of Cu as $CuCl_2$ on respiration (●), photosynthesis (○), light-driven hydrogen production (□), and growth (△) in *Anabaena cylindrica*.

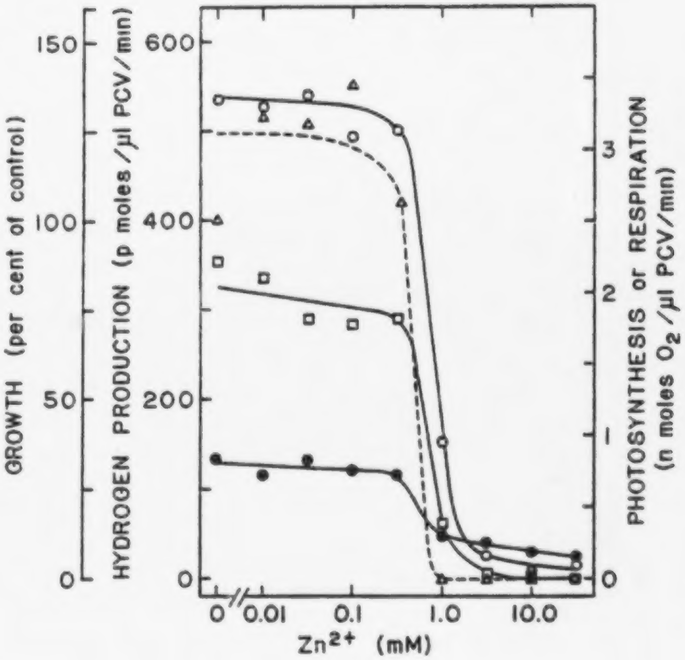


Figure 23.19. The effect of Zn as $ZnCl_2$ on respiration (●), photosynthesis (○), light-driven hydrogen production (□), and growth (△) in *Anabaena cylindrica*.

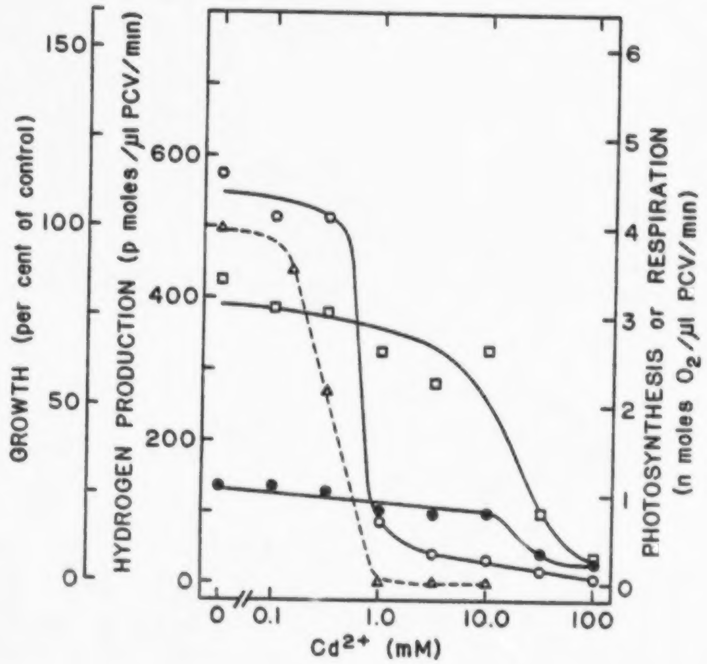


Figure 23.20. The effect of Cd as $CdCl_2$ on respiration (●), photosynthesis (○), light-driven hydrogen production (□), and growth (△) in *Anabaena cylindrica*.

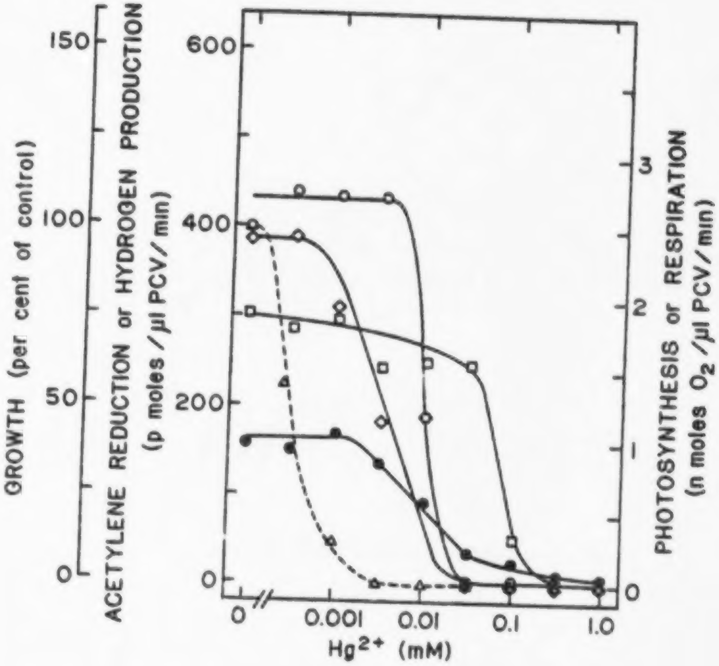


Figure 23.21. The effect of Hg as $HgCl_2$ on respiration (●), photosynthesis (○), light-driven hydrogen production (□), light-driven acetylene reduction (◇), and growth (△) in *Anabaena cylindrica*.

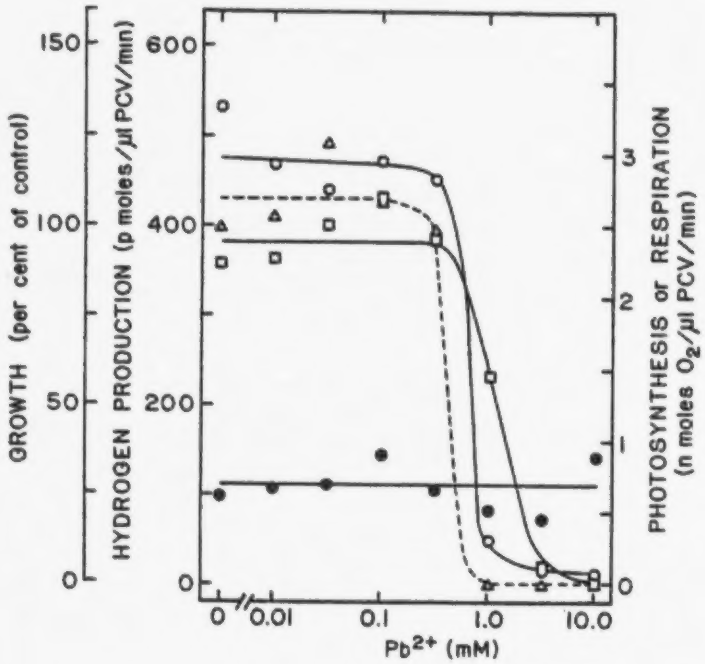


Figure 23.22. The effect of Pb as lead acetate on respiration (●), photosynthesis (○), light-driven hydrogen production (□), and growth (▲) in *Anabaena cylindrica*.

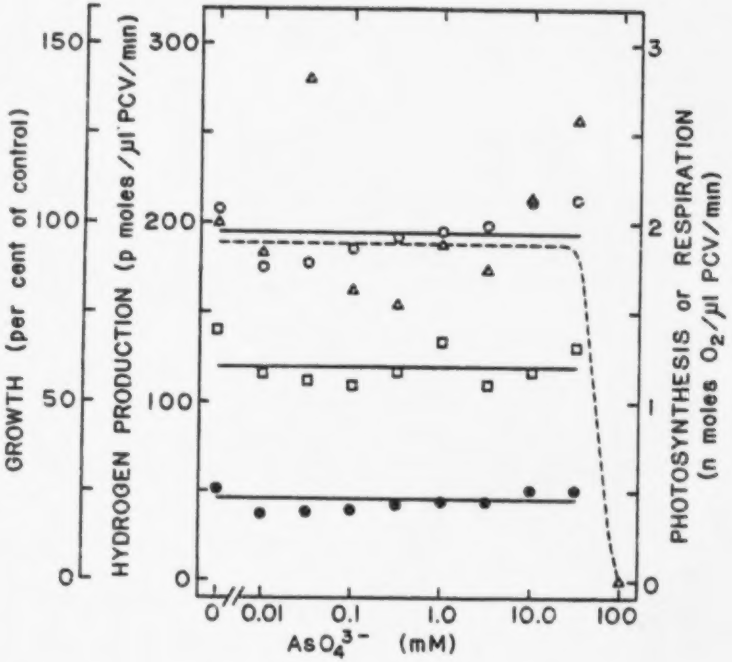


Figure 23.23. The effect of As as Na_2HAsO_4 on respiration (●), photosynthesis (○), light-driven hydrogen production (□), and growth (△) in *Anabaena cylindrica*.

was growth less sensitive to a trace element than any of the measured physiological processes. From Figure 23.27 it may be seen that these same elements cause growth inhibition at generally lower concentrations. Of the elements tested Cr, Ni, Mn, Cu, Sr, and Hg elicited much greater sensitivity in the growth assay. Notable is the dramatic difference in sensitivity seen with Cr which is nearly three hundred times more active in growth studies than in short-term physiological assays. This difference results in Cr, as dichromate, being the third most poisonous element tested overall, having a toxicity similar to copper. Therefore, in decreasing degree of overall toxicity, Hg, Cu, Cr, Ni, Cd, Zn, and Pb are strongly active at 1 *mM* levels or below with Mn, F, and Li showing large growth inhibition below 3 *mM* levels. Mercury remains by far the most potent poison tested and inhibited growth by at least 50% at approximately 1 *mM* levels. On the other hand, at concentrations as high as 100 mM, Na, Cl, K, and Br showed little or no effect. Apparently there is no adverse effect on *Anabaena* due to high osmolarity of the reaction mixtures or culture media during physiological assays or growth periods.

Effects of Select Elements on Growth of *Salvinia* and *Azolla*

In general, the effects of trace elements on the growth of *Salvinia* and *Azolla* were the same. Consistent with the growth studies on *Anabaena*, the greatest effects were seen in the presence of low concentrations of Hg and Cr, both of which strongly affected the waterferns at 1 *mM* levels. Interestingly, the remaining elements did not show strong inhibition at millimolar levels. From the growth results with *Anabaena*, it would be expected that Cd and Pb would greatly retard growth or kill the plants. The symbiotic relationship between these waterferns and the blue-green algae they harbor possibly provides some protection for the algae which are sensitive when cultured individually. In fact, as was clearly observed in the case of the zinc studies, strong inhibition of green algae contaminants was observed at concentrations above 50 μM, whereas little or no effect was seen on the waterferns.

Inhibition by Select Trace Elements

In general, three types of responses to test trace elements were observed: non-toxic, toxic, and differentially toxic. In the case of the majority of the elements relatively little effect was seen at 10 *mM* levels and below. Salt concentrations of 0.1 *M* and more in certain instances are clearly tolerated by these algae (Figure 23.27). This is consistent with the knowledge that certain *Anabaena* species are very tolerant to highly eutrophic or saline situations (Palmer, 1969). The remainder of the elements clearly exert toxic effects. In fact, Rabinowitch (1945) has suggested that compounds that are inhibitory at levels of 10 *mM* and below were "true poisons" since non-specific secondary effects might occur at higher concentrations. For the purpose of this report, highly toxic elements are considered to be those acting at 1 *mM* or lower concentrations. The highly toxic elements detected in this report were Hg, Cu, Cr, Ni, Cd, Zn, and Pb. Two of these, Hg and Cd, showed clear differential inhibition of photosynthesis and photosynthetic hydrogen production. The remaining elements which show physiological inhibition at 1 *mM* levels and below are Cu, Zn, and Pb (Figures 23.24

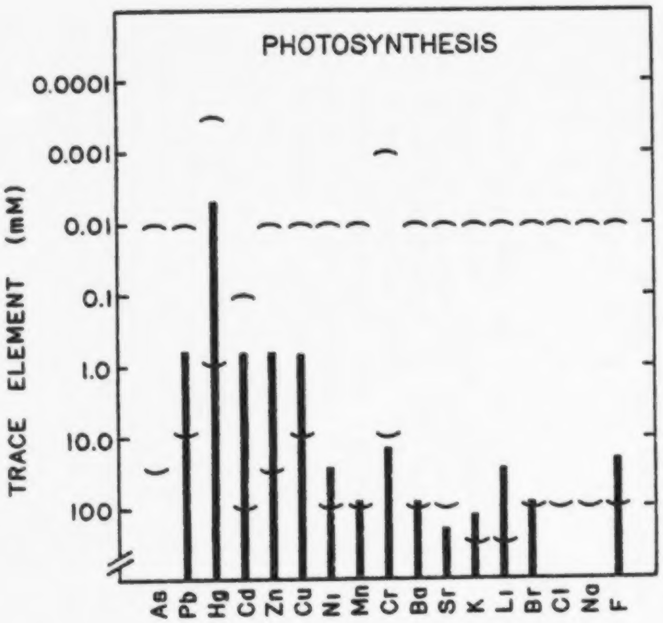


Figure 23.24. Summary of trace element effects on photosynthesis of *Anabaena cylindrica*. Solid bars represent concentrations at which 50% or greater inhibition was observed. Parentheses indicate the concentration range tested.

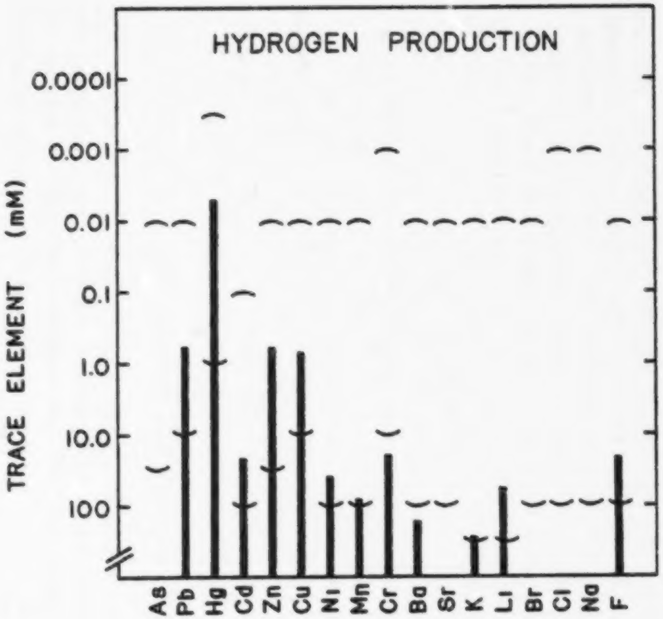


Figure 23.25. Summary of trace element effects on hydrogen production (nitrogen fixation) of *Anabaena cylindrica*. Solid bars represent concentrations at which 50% or greater inhibition was observed. Parentheses indicate the concentration range tested.

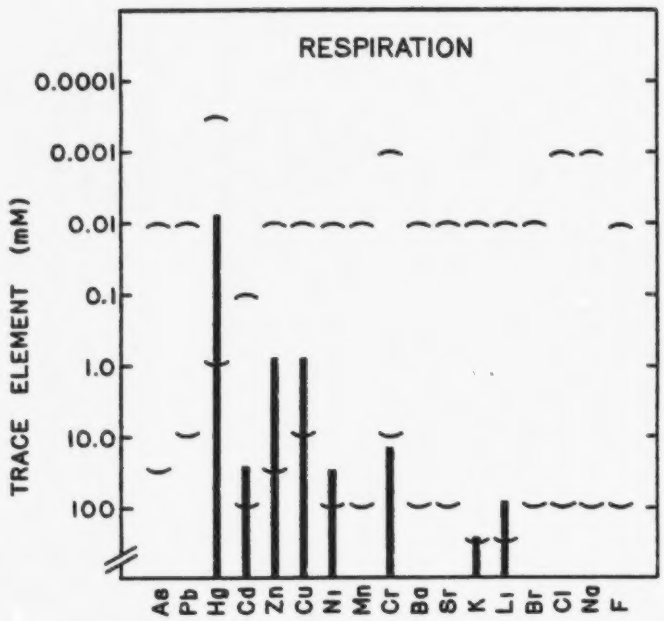


Figure 23.26. Summary of trace element effects on respiration of *Anabaena cylindrica*. Solid bars represent concentrations at which 50% or greater inhibition was observed. Parentheses indicate the concentration range tested.

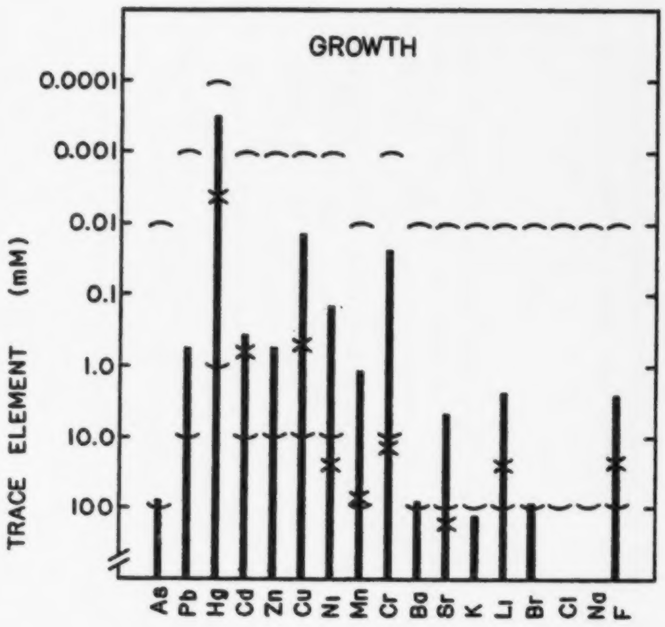


Figure 23.27. Summary of trace element effects on growth of *Anabaena cylindrica*. Solid bars represent concentrations at which 50% or greater inhibition was observed. Parentheses indicate the concentration range tested. The X on each bar indicates the lowest concentration needed to cause 50% inhibition in any of the three physiological assays summarized in Figures 23.24 through 23.26.

to 23.26), and in growth studies Cr and Ni also showed strong toxicity at these levels (Fig. 23.27). These toxic elements and their effects will be discussed in the following paragraphs.

Mercury and Cadmium

Both Hg and Cd are potent inhibitors of algal and higher plant systems (Greenfield, 1942; MacDowal, 1949; Hewitt, 1953; Harriss *et al.*, 1970; Kamp-Nielsen, 1971; Barker,1972; Matsen *et al.*, 1972; Page *et al.*, 1972; Bartlett *et al.*, 1974; Klass *et al.*, 1974; DeFilippis and Pallaghy, 1976; Kayser 1976). In the vegetative cells of blue-green algae complete photosynthesis occurs, including oxygen production, whereas in heterocysts, the sole site of nitrogen fixation in *Anabaena*, only partial photosynthesis occurs and oxygen evolution is absent (Wolk and Wojciuch, 1971; Donze *et al.*, 1972; Kulasooriya *et al.*, 1972). It is also observed that photosynthetic oxygen production is more sensitive to Cd and Hg than photosynthetic hydrogen production (Figures 23.20 and 23.21). Because of the chemical similarities of Cd and Hg, it is not surprising that a similar differential action is seen with both elements. However, Zn, which belongs to the same group of transition metals (IIB) does not demonstrate the same differential effect (Figure 23.19).

The specific effects of Cd and Hg on photosynthesis have been investigated (Izawa and Good, 1969; Gould, 1975; Li and Miles, 1975). The most extensive work has dealt with mercury toxicity. Mercury inhibits photosynthetic electron transport at the site of plastocyanin (Kimimura and Katoh, 1972) but it also causes partial uncoupling of photophosphorylation (Izawa and Good, 1969), and has been reported to be specific for proposed site I phosphorylation (Gould, 1975) but not site II phosphorylation associated with photosystem II. Electron transport has been found to be less sensitive to mercury than phosphorylation. Oxygen evolution or photosynthetic electron transport in vegetative cells is strongly affected by one-tenth the Hg concentration that affects hydrogen evolution from the heterocysts. If mercury is entering the heterocysts as rapidly as the vegetative cells, then electron transport and associated phosphorylation should be inhibited in a manner similar to oxygen production in vegetative cells, assuming plastocyanin to be the site of electron transport inhibition in both cell types. Since respiration is strongly inhibited by 30 μM Hg (Figure 23.21), then the respiratory system of the heterocysts is likely affected. The data can be best explained by the presence of an insensitive photophosphorylation system in heterocysts due to nonspecific competitive, and therefore protective-binding of Hg, or slower transport of mercury to the site of inhibition across the larger heterocyst cell. In any case, it is significant that 30 μM Hg completely inhibits oxygen production and strongly affects respiration, but has little or no effect on hydrogen production (Figure 23.21).

Similar differential inhibition is seen with cadmium at relatively higher concentrations (Figure 23.20). However, it has been reported (Bazzaz and Govindjee, 1974a; Li and Miles, 1975) that Cd inhibits photosynthesis at a site remote from those affected by Hg. Inhibition of electron transport associated with photosystem II is consistent with the inhibition results obtained with both Cd and Hg, whereas the reported sites of Hg inhibition remain inconsistent with the data without invoking other factors.

The pattern of differential inhibition exhibited by Hg and Cd appears similar to that caused by cyanide, wherein oxygen evolution is inhibited at lower concentrations than hydrogen evolution in the absence of oxygen. It is interesting that both cyanide and Hg are known to inhibit photosynthetic electron transport at the site of plastocyanin.

It was observed that mercury inhibition of hydrogen production was more severe in the presence of oxygen. It should be noted that Hg affects oxygen uptake at concentrations of 3 μM and higher in short-term experiments (Figure 23.21). As a result of this respiratory inhibition, the levels of Hg that affect light-stimulated acetylene reduction and light-driven hydrogen evolution are quite different due to oxygen inhibition of nitrogenase activity in the long-term (45 minutes) acetylene reduction assays. In fact, acetylene reduction and respiration exhibited similar inhibition curves (Figure 23.21) and respiration is also more severely affected by Hg than Cd with respect to the inhibition of hydrogen evolution (Figure 23.20). Because of the multiple sites of action of Hg and Cd in biological systems (Passow *et al.*, 1961; Vallee and Ulmer, 1972), it is not surprising to observe that growth is inhibited at one-tenth to one-third the concentrations that retard the most sensitive short-term physiological functions.

Copper

Copper has been used as an algal control chemical since the early 1900's. It inhibits growth at low concentrations, but apparently has a broad range where it has reversible algistatic properties (Fitzgerald and Faust, 1963; Steeman-Nielsen *et al.*, 1969). Copper inhibits growth, photosynthesis, and dark reactions at low concentrations (Greenfield, 1942; MacDowal, 1949; McBrien and Hassall, 1967; Habermann, 1969; Gross *et al.*, 1970; Whitton, 1970; Steeman-Nielsen and Wium-Anderson, 1971; Morris and Russell, 1973; Bartlett *et al.*, 1974; Horne and Goldman, 1974). In the present study, Cu showed strong parallel toxicity of photosynthesis, respiration and nitrogen fixation between 0.3 and 1.0 mM levels, whereas growth was inhibited by nearly 50% at 10 μM Cu (Figure 23.18). An inhibition plateau for growth is seen between 30 and 300 μM $CuCl_2$, and may represent those concentrations which exert algistatic rather than algicidal effects (Fitzgerald and Faust, 1963; Steeman-Nielsen and Kamp-Nielsen, 1970). The inhibitory action of Cu in isolated photosynthetic systems has been recognized by MacDowal (1949), wherein 10 μM Cu clearly inhibits light-driven electron transport by about 50%, and 100 μM levels inhibit the rate between 61 and 93% dependent upon the light intensity. Greenfield's (1942) earlier conclusion was that Cu also strongly affected dark reactions, and subsequently it was suggested that inhibition may be dependent upon anaerobiosis (Hassall, 1962). More recent work on isolated systems has implicated Cu in the inhibition of chloroplast processes, and showed that inhibition was reversed by the addition of Mn^{2+} ions (Habermann, 1969). Later work has indicated that Cu may interact with photosynthetic pigments, and that these interactions are prevented by reducing agents, Mn, and anaerobiosis (Gross *et al.*, 1970). Interesting in this respect is a recent publication reporting the existence of a copper-manganese-protein complex associated with photosystem II (Holdsworth and Arshad, 1977). It appears that no specific site of inhibition can be assigned to Cu.

Lead

Lead toxicity has been of concern in many biological systems (Hewitt, 1953; Vallee and Ulmer, 1972) and it has been shown to inhibit photosynthesis in chloroplasts and algae (Whitton, 1970; Miles *et al.*, 1972; Malavchuk and Gruendling, 1973; Bazzaz and Govindjee, 1974b). In *Anabaena* (Figure 23.22) strong Pb toxicity is observed for all parameters measured at concentrations between 1 and 3 *mM*. Respiration is an exception though it has been shown that Pb inhibits mitochondrial function in higher plants (Koeppe and Miller, 1970). Characteristically, Pb inhibition may involve many binding sites in cells (Vallee and Ulmer, 1972), but inhibition of growth is not seen at concentrations below those in which physiological processes were attenuated. It appears that short-term assays of hydrogen evolution are affected slightly less by 1 *mM* Pb than is photosynthesis. This is consistent with the reported site of Pb inhibition (Bazzaz and Govindjee, 1974b), and resembles the differential inhibition exerted by Cd and Hg.

Chromium, Nickel and Zinc

Chromium, nickel and zinc all exhibited strong toxicity toward *Anabaena* (Figures 23.15, 23.17, 23.19 and 23.27). Chromium toxicity has been recognized in plant systems for some time, and is often an important component in some barren soils (Robinson *et al.*, 1935; Hewitt, 1953; Hunter and Vergano, 1953; DeKock, 1956; Soane and Saunder, 1959). Work has been continued to define Cr toxicity (Turner and Rust, 1971; Wallace *et al.*, 1976), but relatively little information appears to be available concerning toxicity in algae (Leland and Wilkes, 1977). Hexavalent chromium has exhibited greater toxicity to plants than trivalent chromium (Hewitt, 1953), and chromium was used in the dichromate form ($Cr_2O_7^{2-}$) in this study. It can be noted in Figure 23.15 that no toxic effects of dichromate are observed on physiological processes until 10 mM concentrations are applied. However over 50% inhibition of growth is observed when *Anabaena* is supplied with 30 μM dichromate. Thus it appears to demonstrate toxicity similar to copper. In growth assays with *Azolla* and *Salvinia*, micromolar concentrations of dichromate were also extremely toxic.

Zinc and nickel are recognized to be toxic to higher plants (Baker, 1972; Hewitt, 1953; Hunter and Vergano, 1953; DeKock, 1956) and algae (Greenfield, 1942; Whitton, 1970; Coleman *et al.*, 1971; Bartlett *et al.*, 1974; Rachlin and Farran, 1974; Greene *et al.*, 1975; DeFilippis and Pallaghy, 1976). In *Anabaena*, Zn effects were close to maximal at 1 *mM* for all parameters tested including growth which did not show appreciably greater sensitivity (Figure 23.19). However, Ni toxicity exhibited a much different pattern. Nickel at 1 *mM* had little or no effect on any physiological process that was tested, but was completely inhibitory to growth (Figure 23.17). A slow decrease in the activity of the physiological parameters was seen as Ni concentrations were increased from 1 to 100 *mM* levels. As contrasted to the Zn experiments, Ni shows a great differential in toxicity between growth and short-term physiological processes.

CONCLUSIONS

The effects of selected trace elements emitted from coal-fired power plants on nitrogen fixation, photosynthesis, respiration and growth were surveyed in the blue-green algae, *Anabaena cylindrica*. The seventeen elements tested were F, Na, Cl, Br, Li, K, Sr, Ba, Cr, Mn, Ni, Cu, Zn, Cd, Hg, Pb, and As. In order of decreasing toxicity, it was found that Hg, Cu, Cr, Ni, Cd, Zn, and Pb exerted inhibition at levels of 1 *mM* or below. In short-term physiological experiments Hg, Cu, Zn, Cd, and Pb exhibited toxicity to biological functions at 1 *mM* or lower levels. The algae showed much greater sensitivity to Cr, Ni, Mn, Cu, Sr, and Hg in the long-term growth studies than in the short-term physiological experiments. Inhibition of *Azolla* growth was strong at 1 *mM* Hg and Cr but was insensitive to millimolar concentrations of Pb, Zn, Cd, and Sr. Chromium is therefore identified as an extremely toxic element in both systems. In *Anabaena*, differential inhibition of nitrogen fixation, photosynthesis, respiration, and growth was observed with varied concentrations of several elements. The metabolic inhibitors KCN, DCMU, *m*-Cl-CCP, and DBMIB were studied, and the interaction of oxygen and light on nitrogenase activity was determined, with implications for field and laboratory nitrogen fixation studies. Under certain conditions hydrogen production serves as a sensitive measure of nitrogen fixation.

REFERENCES

Barker, W. G. 1972. Toxicity Levels of Mercury, Lead, Copper, and Zinc in Tissue Culture Systems of Cauliflower, Lettuce, Potato, and Carrot. Can. J. Bot., 50:973-976.

Bartlett, L., F. W. Rabe, and W. H. Funk. 1974. Effects of Copper, Zinc, and Cadmium on *Selanastrum capricornutum*. Water Res., 8:179-185.

Bazzaz, M. B. and Govindjee. 1974a. Effects of Cadmium Nitrate on Spectral Characteristics and Light Reactions of Chloroplasts. Envir. Letters, 6:1-12.

Bazzaz, M. B. and Govindjee. 1974b. Effects of Lead Chloride on Chloroplast Reactions. Envir. Letters, 6:175-191.

Biggins, J. 1969. Respiration in Blue-green Algae. J. Bact., 99:570-575.

Bishop, N. I. 1958. The Influence of the Herbicide, DCMU, on the Oxygen-Evolving System of Photosynthesis. Biochim. Biophys. Acta, 27:205-206.

Brown, A. H. and G. C. Webster. 1953. The Influence of Light on the Rate of Respiration of the Blue-green Alga *Anabaena*. Am. J. Bot., 40:753-758.

Carr, N. G. and B. A. Whitton, eds. 1973. The Biology of Blue-green Algae. Botanical Monographs, vol. 9. Univ. of Calif. Press, Berkeley.

Castenholz, R. W. 1970. Laboratory Culture of Thermophilic Cyanophytes. Schweizerische Zeitschrift für Hydrologie, 32:538-551.

Coleman, R. D., R. L. Coleman, and E. L. Rice. 1971. Zinc and Cobalt Bio-Concentrations and Toxicity in Selected Algal Species. Bot. Gaz., 132:102-109.

Dean, J. A., ed. 1973. Lang Handbook of Chemistry. 11th edition, McGraw-Hill Book Co., N.Y.

DeFilippis, L. F., and C. K. Pallaghy. 1976. The Effect of Sublethal Concentrations of Mercury and Zinc on *Chlorella pyrenoidosa*. I. Growth Characteristics and Uptake of Metals. Z. Pflanzenphysiol., 78:197-207.

DeKock, P. C. 1956. Heavy Metal Toxicity and Iron Chlorosis. Ann. Bot., 20:133-141.

Donze, M., J. Haveman, and P. Schiereck. 1972. Absence of Photosystem 2 in Heterocysts of the Blue-green Alga *Anabaena*. Biochim. Biophys. Acta, 256:157-161.

Drozd, J., and J. R. Postgate. 1970. Interference by Oxygen in the Acetylene Reduction Test for Aerobic Nitrogen Fixing Bacteria. J. Gen. Microbiol., 60:427-429.

Fitzgerald, G. P., and S. L. Faust. 1963. Factors Affecting the Algicidal and Algistatic Properties of Copper. Appl. Microbiol., 11:345-351.

Fogg, G. E., W. D. P. Stewart, P. Fay, and A. E. Walsby. 1973. The Blue-green Algae. Academic Press, N.Y.

Gould, J. M. 1975. The Phosphorylation Site Associated with the Oxidation of Exogenous Donors of Elecrons to Photosystem I. Biochim. Biophys. Acta, 387:135-148.

Greene, J. C., W. E. Mitler, T. Shiroyama, and E. Merwin. 1975. Toxicity of Zinc to the Green Algae *Selenastrum capricornutum* as a Function of Phosphorus or Ionic Strength. In: Proc. Biostimulation-Nutrient Assessment Workshop (16-17 Oct. 1973), Ecol. Res. Ser. 660/3-75-034, U.S. Envir. Prot. Agency, Corvallis, OR. pp. 28-43.

Greenfield, S. S. 1942. Inhibitory Effects of Inorganic Compounds on Photosynthesis in *Chlorella*. Am. J. Bot., 29:121-131.

Gross, R. E., P. Pugno, and W. M. Dugger. 1970. Observations on the Mechan of Copper Damage in *Chlorella*. Plant Physiol., 46:183-185.

Haaker, H., and C. Veeger. 1977. Involvement of the Cytoplasmic Membrane i Nitrogen Fixation by *Azotobacter vinelandii*. Eur. J. Biochem., 77:1-10

Habermann, H. M. 1969. Reversal of Copper Inhibition in Chloroplast Reactions by Manganese. Plant Physiol., 44:331-336.

Harriss, R. C., D. B. White, and R. B. Macfarlane. 1970. Mercury Compounds Reduce Photosynthesis by Plankton. Science, 170:736-737.

Hassall, K. A. 1962. Uptake of Copper and its Physiological Effects on *Chlorella vulgaris*. Physiol. Plant., 16:323-332.

Henricksson, E. 1971. Algal Nitrogen Fixation in Temperate Regions. In: Biological Nitrogen Fixation in Natural and Agricultural Habitats, T. A. Lie and E. G. Mulder, eds. Plant Soil (Special Volume), pp. 415-419.

Hewitt, E. J. 1953. Metal Interrelationships in Plant Nutrition. I. Effects of Some Metal Toxicities on Sugar Beet, Tomato, Oat, Potato, and Marrowstem Kale Grown in Sand Culture. J. Exp. Bot., 4:59-64.

Heytler, P. G., and W. W. Prichard. 1962. A New Class of Uncoupling Agents - Carbonyl Cyanide Phenylhydrazones. Biochem. Biophys. Res. Comm., 7:272-275.

Holdsworth, E. S., and J. H. Arshad. 1977. A Manganese-Copper-Pigment-Protein Complex Isolated from the Photosystem II of *Phaeodactylum tricornutum*. Arch. Biochem. Biophys., 183:361-373.

Horne, A. J., and C. R. Goldman. 1974. Suppression of Nitrogen Fixation by Blue-green Algae in a Eutrophic Lake with Trace Additions of Copper. Science, 183:409-411.

Hunter, J. G., and O. Vergnano. 1953. Trace Element Toxicity in Oat Plants. Ann. Appl. Biol., 40:761-775.

Izawa, S., and N. E. Good. 1969. Effect of p-chloromercuribenzoate (PCMB) and Mercuric Ion on Chloroplast Photophosphorylation. Prog. Photosyn. Res., 3:1288-1298.

Jones, L.W., and N.I. Bishop. 1976. Simultaneous Measurement of Oxygen and Hyrdrogen Exchange from the Blue-Green Algae *Anabaena*. Plant Physiol., 57(4):659-665.

Kamp-Nielsen, L. 1971. The Effect of Deleterious Concentrations of Mercury on the Photosynthesis and Growth of *Chlorella pyrenoidosa*. Physiol. Plant., 24:556-561.

Kayser, H. 1976. Waste-water Assay with Continuous Algal Cultures: the Effect of Mercuric Acetate on the Growth of Some Marine Dinoflagellates. Marine Biol., 36:61-72.

Kimimura, M., and S. Katoh. 1972. Studies on Electron Transport Associated with Photosystem I. I. Functional Site of Plastocyanine; Inhibitory Effects of $HgCl_2$ on Electron Transport and Plastocyanine in Chloroplasts. Biochim. Biophys. Acta, 283:279-292.

Kimimura, M., S. Katoh, I. Ikegami, and A. Takamiya. 1971. Inhibitory Site of Carbonylcyanide *m*-chlorophenylhydrazone in the Electron Transfer System of the Chloroplast. Biochim. Biophys. Acta, 234:92-102.

Klass, E., D. W. Rowe, and E. J. Massaro. 1974. The Effect of Cadmium on Population Growth of the Green Algae *Scenedesmus quadracauda*. Bull. Environ. Contam. Toxicol., 12:442-445.

Koeppe, D. E., and R. J. Miller. 1970. Lead Effects on Corn Mitochondrial Respiration. Science, 167:1376-1378.

Kulasooriya, S. A., N. J. Lang, and P. Fay. 1972. The Heterocysts of Blue-green Algae. III. Differentiation and Nitrogenase Activity. Proc. Roy. Soc. B, 181:199-209.

Leland, H. V., and D. J. Wilkes. 1977. Heavy Metals and Related Trace Elements. J. Water Pollut. Control Fed., 49:1340-1369.

Li, E. H., and C. D. Miles. 1975. Effects of Cadmium on Photoreaction II of Chloroplasts. Plant Sci. Lett., 5:33-40.

Loomis, A. G. 1928. Solubilities of Gases in Water. In: International Critical Tables 3, E. W. Washburn, ed. McGraw-Hill Book Company, Inc., N.Y. pp. 255-261.

MacDowall, F. D. H. 1949. The Effects of Some Inhibitors of Photosynthesis Upon the Photochemical Reduction of a Dye by Isolated Chloroplasts. Plant Physiol., 24:462-480.

MacRae, I. C., and T. F. Castro. 1967. Nitrogen Fixation in Some Tropical Rice Soils. Soil Sci., 103:277-280.

Malanchuk, J. L., and G. K. Gruendling. 1973. Toxicity of Lead Nitrate to Algae. Water Air Soil Pollut., 2:181-190.

Matsen, R. S., G. E. Mustoe, and S. B. Chang. 1972. Mercury Inhibition on Lipid Biosynthesis in Freshwater Algae. Environ. Sci. Technol., 6:158-160.

Mayland, H. F., T. H. McIntosh, and W. H. Fuller. 1966. Fixation of Isotopic Nitrogen on a Semiarid Soil by Algal Crust Organisms. Soil Sci. Soc. Am. Proc., 30:56-60.

McBrien, D. C. H., and K. A. Hassall. 1967. The Effect of Toxic Doses of Copper Upon Respiration, Photosynthesis and Growth of *Chlorella vulgaris*. Physiol. Plant., 20:113-117.

Miles, C. D., J. R. Brandle, D. J. Daniel, O. Chu-Der, P. D. Schnare, and D. J. Uhlik. 1972. Inhibition of Photosystem II in Isolated Chloroplasts by Lead. Plant Physiol., 49:820-825.

Montana State Department of Natural Resources and Conservation. 1974. Draft Environmental Impact Statement on Colstrip Electric Generating Units 3 and 4. Vol. 3A. Helena. pp. 215-233.

Morris, O. P., and G. Russell. 1973. Effect of Chelation on Toxicity of Copper. Marine Pollut. Bull., 4:159-160.

Ouitrakul, R., and S. Izawa. 1973. Electron Transport and Photophosphorylation as a Function of the Electron Acceptor. II. Acceptor-specific Inhibition by KCN. Biochim. Biophys. Acta, 305:105-118.

Page, A., F. Bingham, and C. Nelson. 1972. Cadmium Absorption and Growth of Various Plant Species as Influenced by Solution Cadmium Concentration. J. Environ. Qual., 1:288-292.

Palmer, C. M. 1969. A Composite Rating of Algae Tolerating Organic Pollution. J. Phycol., 5:78-82.

Passow, H., A. Rothstein, and T. W. Clarkson. 1961. The General Pharmacology of the Heavy Metals. Pharmacol. Rev., 13:185-224.

Rabinowitch, E. I. 1945. Photosynthesis and Related Processes. Vol. I, Chemistry of Photosynthesis, Chemosynthesis and Related Processes *in vitro* and *in vivo*. Interscience Publishers, Inc. N.Y. p. 335.

Rachlin, J. W., and M. Farran. 1974. Growth Response of the Green Algae *Chlorella vulgaris* to Selective Concentrations of Zinc. Water Res., 8:575-577.

Rivera-Ortiz, J. M., and R. H. Burris. 1975. Interactions Among Substrates and Inhibitors of Nitrogenase. J. Bact., 123:537-545.

Robinson, W. D., G. Edgington, and H. G. Byers. 1935. Chemical Studies of Infertile Soils Derived from Rock High in Magnesium and Generally High in Chromium and Nickel. Tech. Bull. 471, USDA. pp. 1-29.

Senger, H., and H. J. Wolf. 1964. Eine Automatische Verdünnungsanlage und Ihre Anwendung zur Erzielung Homokontinuirlicher *Chlorella*-Kulturen. Arch. Mikrobiol., 48:81-94.

Soane, B. D., and D. H. Saunder. 1959. Nickel and Chromium Toxicity of Serpentine Soils in Southern Rhodesia. Soil Sci., 88:322-330.

Steeman-Nielsen, E., L. Kamp-Nielsen, and S. Wium-Andersen. 1969. The Effect of Deleterious Concentrations of Copper on the Photosynthesis of *Chlorella pyrenoidosa*. Physiol. Plant., 22:1121-1133.

Steeman-Nielsen, E., and L. Kamp-Nielsen. 1970. Influence of Deleterious Concentrations of Copper on the Growth of *Chlorella pyrenoidosa*. Physiol. Plant., 23:828-840.

Steeman-Nielsen, E., and S. Wium-Andersen. 1971. The Influence of Cu on Photosynthesis and Growth in Diatoms. Physiol. Plant, 24:480-484.

Tetley, R. M., and N. I. Bishop. 1977. Oxygen Stimulation of H_2 Production in a Blue-green Algae. Plant Physiol., 59(suppl.):130.

Trebst, A., E. Harth, and W. Draber. 1970. On a New Inhibitor of Photosynthetic Electron-Transport in Isolated Chloroplasts. Z. Naturforsch., 25b:1157-1159.

Turner, M. A., and R. H. Rust. 1971. Effect of Chromium on Growth and Mineral Nutrition of Soybeans. Soil Sci. Am. Proc., 35:755-758.

Vallee, B. L., and P. D. Ulmer. 1972. Biochemical Effects of Mercury, Cadmium and Lead. Ann. Rev. Biochem., 41:91-128.

Vernon, L. P. 1960. Spectrophotometric Determination of Chlorophylls and Pheophytins in Plant Extracts. Anal. Chem., 32:1144-1150.

Wallace, A., S. M. Soufi, J. W. Cha, and E. M. Romney. 1976. Some Indirect Effects of Chromium Toxicity on Bush Bean Plants Grown in Soil. Plant Soil, 44:471-473.

Webster, G. C., and A. W. Frenkel. 1953. Some Respiratory Characteristics of the Blue-green Algae, *Anabaena*. Plant Physiol., 28:63-69.

Whitton, B. A. 1970. Toxicity of Zinc, Copper and Lead to Chlorophyta from Flowing Waters. Arch. Mikrobiol., 72:353-360.

Wolk, C. P., and E. Wojciuch. 1971. Photoreduction of Acetylene by Heterocysts. Planta, 97:126-134.

SECTION 24

THE USE OF REMOTE SENSING IN EVALUATING SO_2 DAMAGE TO GRASSLANDS

J. E. Taylor, W. C. Leininger,
and T. R. Osberg

ABSTRACT

Aerial photography in the vicinity of the EPA's southeastern Montana grassland research sites has been collected since 1974. This includes conventional coverage acquired from several federal agencies and original large scale imagery flown by the project investigators. A system using small aircraft and 70mm cameras has been developed and used in periodic monitoring of the study sites and adjacent locations. Color, color infrared (CIR) and black-and-white films are exposed at scales ranging from 1:3700 to 1:30,000 for various purposes. In 1977, four fixed wing flights and one helicopter photo mission were flown. The resultant imagery was analyzed through visual interpretation, color standard matching, image enhancement, and densitometric analysis. The latter yields numeric data which can be related to various kinds of ground data by regression techniques. Highly significant relationships were observed between image densities and SO_2 treatment levels on the fumigation plots. Leaf senecense of western wheatgrass showed similar tendencies. Color infrared taken during the active growth season gave the best results. ZAPS II shows poorer relationships than ZAPS I because of its higher inherent variability in microtopography and plant community patterns. The influences of grazing deferment, grasshopper infestations, and annual climatic fluctuations are discussed in relation to data interpretation and the necessity for long-term ecological studies in grasslands. Vegetation maps have been prepared for the ZAPS sites.

INTRODUCTION

The background and objectives of our overall project are given in the Introduction to Section 3. Here, we discuss procedures and results of aerial and ground photography which we have developed to monitor air pollution effects in a grassland ecosystem.

MATERIALS AND METHODS

Aerial Photography

Procedures

In our aerial photography we have concentrated on developing procedures which use standard, readily available equipment, since if a system is to be applied in a variety of situations it must not necessitate highly specialized, costly investments.

The aircraft and camera system we use (Cessna 182, Hasselblad EL/M camera) is described in Section 3. This is essentially an "off-the-shelf" system, with the exception of a belly port in the aircraft, fitted with a custom-built camera mount.

We use color positive, color negative, color infrared (CIR), and black-and-white films for various purposes. Color positive and color infrared (Kodak 2448 and 2443, respectively) both yield transparencies which can be viewed on a light table or used to make paper prints. We use the Cibachrome process for prints because of its sharpness, good color saturation, and resistance to fading. Where we want prints only, we use color negative film, usually Kodak Vericolor VPS-120. Any of these films can also be used to make black-and-white prints. However, when we want extremely sharp black-and-whites for mapping or other purposes requiring high resolution we use H&W VTE film.

In 1974 and 1975, when we were first working out the details of procedures, we used both 70mm (120 roll film) and 35mm film formats. The 35mm material was easily processed immediately after each mission so we could check on exposure and target centering. Also, we could go into the field the next day and compare ground features with their photographic images. The exposure was a particularly difficult problem because of the great sensitivity of color infrared to variations in cloud cover and shadows.

With experience under a variety of light conditions and an accumulation of ground truth data from the study sites, we have learned to adjust exposure to the situation, and have found that immediate ground checking is not necessary on familiar targets. For this reason, we now use the 70mm format most of the time, since it covers almost five times the area per frame as 35mm at the same photo scale. Also, the 70mm casette allows 70 exposures without changing camera backs, a great advantage in the air. 35mm CIR is readily available only in 20 exposure casettes.

Our workhorse film type is color infrared. This material has good sharpness, penetrates light haze very well, and most importantly, is sensitive to subtle differences in plant species' physiological states, particularly their cell water contents. For any particular growth stage, infrared reflectance is characteristic of each species, and also is strongly influenced by stress factors.

We use various photo scales, depending upon the amount of ground detail (resolution) and the area per frame (coverage) required for different purposes. All of our vertical aerial photography is flown with 60% end lap so it can be viewed stereoscopically.

Our largest scale is 1:3700, which is obtained by flying at 305 meters above ground with an 80mm lens. Larger scales are effectively precluded by the minimum 1/500 second shutter speed of the camera. At 195 km per hour, an object on the ground moves 10.5 cm (relative to the camera), during exposure. The resultant image motion causes a slight displacement in the direction of flight. This is negligible at smaller scales, but becomes noticeable at lower elevations. This phenomenon can be seen in the ZAPS photos, where the pipes oriented at right angles to the flight direction appear to be slightly thicker than those parallel to flight.

Even with image displacement, photography at this large scale shows excellent ground detail, discriminating individuals of many plant species and even 10 x 15 cm plastic marking flags and the wires supporting them.

When more coverage is desired and ground detail is less critical, flying altitude is increased or lenses changed to shorter focal lengths. For instance, to get each ZAPS location in one frame we use a scale of 1:15,000. This still allows good species discrimination and also reveals more of the surrounding areas for topographic analysis, drift detection, etc.

Our smallest practical scale is about 1:30,000, approximately two inches per mile. This is obtained by flying about a mile (1609 meters) above the ground with a 50mm lens. This gives excellent synoptic coverage, shows land use patterns, generalized vegetation and topography, etc.

With repeated aerial photography throughout the growing season, we propose to characterize the normal sequence of plant signatures, and then detect any deviations in this normal pattern which might be brought about by such stresses as plant disease, drought, insect infestations, or air pollution.

With color materials of any kind, we have observed variation in color from one batch to another. This can confound attempts to distinguish species signatures from normal variation in film. A good share of this problem can be eliminated by standardizing techniques as much as possible. We buy film in sufficient quantities so that all the photography from a growing season has the same emulsion number. Film is kept frozen until it is used, then cool until processing, which is done as soon as possible after each mission.

Film is processed by a commercial firm which specializes in aerial photographic work. A density step wedge is printed onto the film end before processing for density and color calibration.

For optimum discrimination of infrared reflectance, CIR film requires a minus blue filter, which removes some of the shorter wave length radiation to which the film is also sensitive. The manufacturer's recommendation is a yellow (Wratten 12) filter. We prefer a Wratten 15, which is a little more orange colored, and which cuts off slightly more of the yellow-orange portion of the spectrum. In our situation, this improves the separation of infrared reflectances, which are rendered on CIR in tones of red. Also, it produces ground tones which are less blue than those obtained with the 12 filter, and we find these more visually pleasing.

Most of our photography is accomplished with lenses of 50 or 80mm, but we sometimes use 150mm and 250mm lenses for increasing scale without reducing elevation. For any given scale, the use of a longer lens at higher elevation decreases the effect of vertical scale displacement because of ground relief. However, it is harder to accurately sight at higher elevations, so if there is little margin for tracking error, it is better to fly lower with a shorter lens.

Analysis of Photography

Visual Observation

The simplest kind of photo interpretation (PI) is identification on the photography of ground objects. Shape, size, shadows, texture, and proximity to other objects all are clues to PI, but in color systems, (including CIR) color is the most conspicuous and useful characteristic in detecting both temporal and spatial changes.

Our earliest procedure for cataloging colors was to simply assign subjective names based on the color perception of the observer. This suffers from the serious disadvantages of being non-quantitative, being influenced by the colors of adjacent objects and ambient light, and numerous other problems.

We reduced the subjectivity of color description by employing the Munsell Color System (Munsell Corporation, 1976). Standard color chips of known hue, chroma, and value are matched with the photograph under standard light conditions (Anderson et al., 1977; Anderson, 1978). This approach has proven to be repeatable among workers, although it is less so as colors approach grey. Unfortunately, the color designations are not coded in a way which allows them to be correlated with other quantitative data.

Color Enhancement

We have analyzed some of our photography with a scanning densitometer. This device displays the various density levels of the scene on a cathode ray tube. Each density step can be assigned a color, making subtle differences in the original photography much more conspicuous. On the ZAPS plots, this has indicated a trend in decreased density with increased SO_2 since the first growing season.

The output still is subjective because the density ranges are under the control of the operator, and we have not had access to a calibrated system. Thus, while revealing interesting features of the scene, as well as problems in uniformity over the surface of the photography caused by filter cut-off and vignetting, this color enhancement has not been highly useful in providing data for further analysis.

Densitometric Analysis

Our previous limitations in obtaining numerical data from our aerial photography have been partially resolved with our recent access to a transmission densitometer. We used a Macbeth TD-504 with Status A filtration belonging to the EPA Environmental Photo Interpretation Center (EPIC) to examine some of our 1977 coverage from the ZAPS and Colstrip sites. This instrument measures the density of the red, green, and blue information content of film, using a 3mm aperture. The optical density of each color is displayed digitally. These values or ratios between them can be used to develop correlations with other kinds of digital data from the ground.

We have run correlations with SO_2 treatment rates, leaf senescence data for western wheatgrass (Dodd et al, Section 12), and livestock grazing effects.

Calspan Report

The October, 1975, 1:55,000 CIR was flown over the study sites by NASA. The portion of this coverage which included ZAPS I and considerable surrounding country was examined by Schott et al. (ND), using a patented density ratioing procedure developed at Calspan Corporation (Piech et al., 1974). Their analysis indicated some patterns of SO_2 drift and accumulation at several spots some distance from the funigation plots. Our photography and ground studies have not corroborated these observations, but we are continuing to monitor the locations, since the Calspan procedure is reported to be more sensitive to low levels of stress than conventional methods. It may be that we simply have not detected their patterns yet.

Vegetation Mapping

Using CIR from the active growing season (June), plant communities and some conspicuous species on both ZAPS plots were delineated on overlays. This mapping was done while traversing the area with photo prints in hand. The mapping units were defined as they were encountered. There were 104 vegetational units and five others. Plant community names are based on the predominant species influencing the photo signatures.

RESULTS AND DISCUSSION

Aerial Photography

During 1977, we flew four photo missions over the ZAPS sites and three in the vicinity of Colstrip. The photography obtained was color infrared and color, at scales from 1:3700 to 1:30,000. In August we flew a helicopter mission around Colstrip at 1:900 to 1:2700. A list of our 1977 aerial photography is given in Appendix 24.1. The smaller scale photography of the study sites, which is held by EPA/EPIC, is listed in Appendix 24.2.

Analysis of Photography

Visual Observation

Ever since the end of the first growing season in 1974, we have observed a trend of increased reflectance across the ZAPS plots. This has appeared in both color and CIR, although it is more pronounced in the latter. Corroborating ground data have not been forthcoming. Much of the apparent "pollution" effect appears to have been a bleaching of plant tissues, particularly western wheatgrass. Production, phenology, or plant species diversity have not supported the hypothesis that permanent damage was occuring with the SO_2 fumigation, at lease for the first few years. This has been considered by some to be a problem of the remote sensing rather than reflecting inadequacy in the sensitivity of ground data. In 1977, supportive data have finally started to appear. This is discussed in detail in Section 16 and below under "Densitometric Analysis". This suggests that the aerial imagery can in fact detect early changes in growth patterns of native grassland species before overt signs of stress are visible on the ground.

Munsell Color System

The Munsell Color System is used in a MS Thesis (Anderson, 1978). We have found that color matching is quite feasible, and that differences among interpreters are relatively slight until color saturations decrease to near-grey levels. If viewed under constant light conditions, the standard color chips and colors of objects in aerial photographs can be accurately matched if the photo colors are isolated from surrounding colors with neutral grey cards. The main drawback to this kind of color description is the problem of avoiding color shifts when making paper prints from color transparencies. (The color chips cannot readily be compared with the original transparencies because the chips are opaque, so one is faced with the difficulty of comparing a reflected image with a transmitted one, and color matches are very hard to make.)

Densitometric Analysis

As previously mentioned, the densitometer we use has a viewing aperture of 3mm. This makes photo scale very important in density measurements, since even at our largest scale (1:3700), the area being measured represents a little over 96 m^2. Thus, density readings integrate the reflectance characteristics of various plant species as well as bare ground, rock, litter, and anything else occupying space in the scene. At large scale, subsamples of image density can be taken within plots the size of ZAPS. At our smaller scales, each density value represents the sum of reflectances for an entire plot. The correlations discussed below are all based on subsampled large-scale photography.

We examined several kinds of ground information as related to the color densities in both color and color infrared film. May and June exposures were included. Density ratios were examined, but were consistently unrelated to ground data, so are not discussed.

The relationship between optical density and ZAPS treatments is shown in Figure 24.1. There is a general decrease in density with increasing SO_2. This relationship is more linear with ZAPS I, especially when based on CIR imagery. On ZAPS II CIR, the low treatment has a greater than expected density, particularly green and blue. The reasons are that this plot has the least bare ground and the greatest vegetational cover of any of the plots. Further, forbs contribute a higher percentage of the canopy than on any other treatment. Since CIR is recording active plant tissue, the observed pattern makes sense.

With the color film, the ZAPS I medium plot also has a displaced density curve in May. This may be due to the high amount of bare soil on the plot, which is recorded dark when the soil is moist in early season.

In Figure 24.2. we have regressed density on SO_2 levels using color infrared film. We used the theoretical values of 0, 2, 5, and 10pphm for the control, low, medium, and high SO_2 levels, respectively. The actual fumigation rates deviated slightly from the theoretical levels. Observed values are discussed in Sections 9 and 10. This shows the close correlation on ZAPS I between SO_2 rate and color density. In CIR, the red density (which includes the infrared record) is consistently the most closely related to stress levels. In June, the month of maximum plant growth, the better curve fit is observed. The relatively poor relationship on ZAPS II is attributed to the uneven microtopography. This influences not only plant growth and vigor, but also species composition and canopy cover. This irregular substrate also is evidenced in diversity data (Section 16).

Color film density is related to SO_2 rates in Figure 24.3. The trends follow those seen in CIR, except that the relative densities of the colors are lower. Also, in order of increasing density, the colors rank green, blue, red compared with red, green, blue in CIR. Green, the dominant color of actively growing vegetation, is the least variable indicator of pollution stress.

Leaf senescence of western wheatgrass (Dodd, et al., Section 12), is related to the optical density of CIR in Figure 24.4. In June, the ZAPS I relationship is very good ($r^2 = 0.94 - 0.97$). The ZAPS II fit is disappointing, probably because of inter-site variability and possibly also due to the strongly fumigated "hot spots". The earlier (May) reflectance

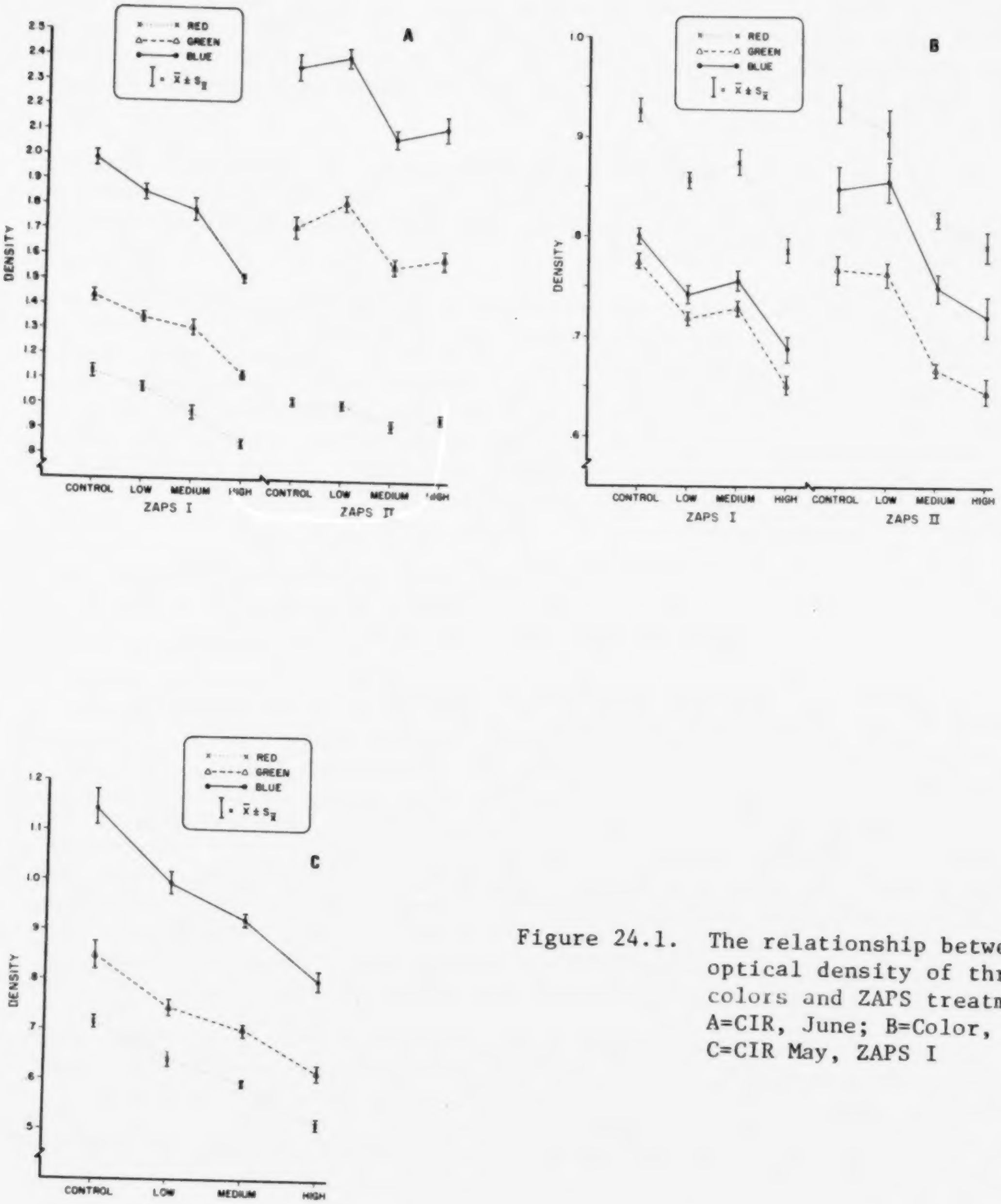


Figure 24.1. The relationship between optical density of three colors and ZAPS treatments. A=CIR, June; B=Color, May; C=CIR May, ZAPS I

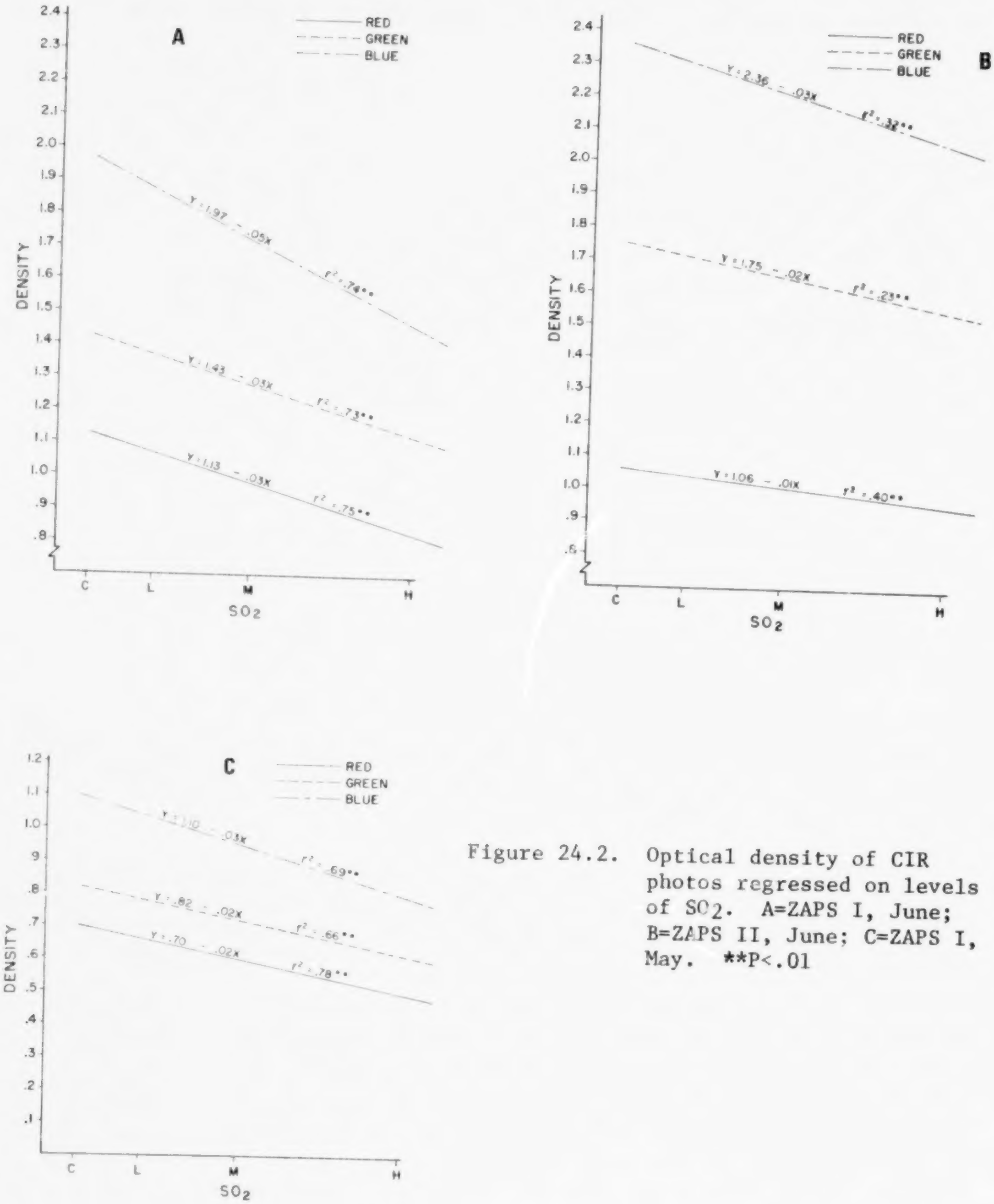


Figure 24.2. Optical density of CIR photos regressed on levels of SO_2. A=ZAPS I, June; B=ZAPS II, June; C=ZAPS I, May. **P<.01

sample may include previous year's plant material. The higher leaf senescence observed on the control plot in May is anomalous. It certainly contributes to a lower coefficient of variability with density data.

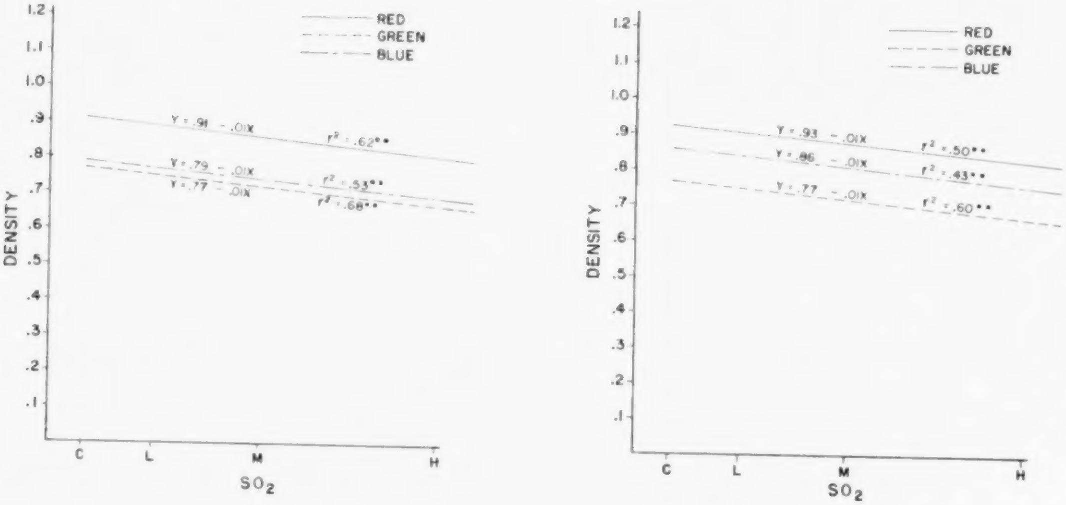


Figure 24.3. Correlation of density of May color photography of ZAPS I (left) and ZAPS II (right) to levels of SO_2 stress. ** $P < .01$

Density shows a similar relationship to May leaf senescence in color film (Figure 24.5). As before, ZAPS I shows the better correlation. On ZAPS II the leaf data are highly variable. The combination of color film and ZAPS II early observations yields the poorest curve fits noted.

We feel that with color infrared film and peak-of-green, large-scale photography, we are detecting real differences in treatments. We have ground data to explain the remote sensing indications, and now we have techniques to relate ground and aerial information. We will extend this line of research in the coming season and will retrospectively examine data from previous years. The remote sensing aspect of this project started small, but it was large enough to record annual data. It appears now that it will play a key role in the siting and monitoring protocol.

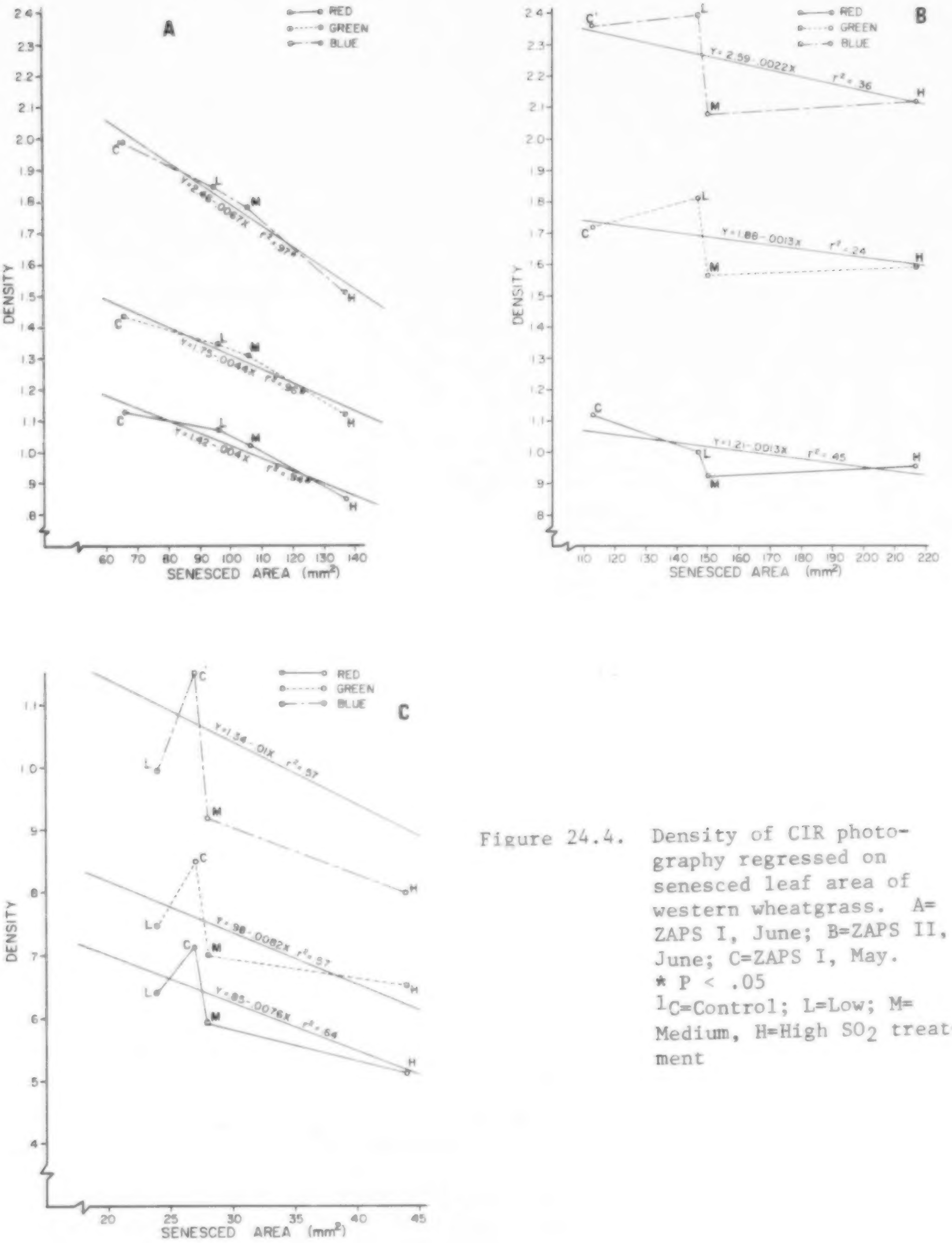


Figure 24.4. Density of CIR photography regressed on senesced leaf area of western wheatgrass. A= ZAPS I, June; B=ZAPS II, June; C=ZAPS I, May.
* $P < .05$
[1] C=Control; L=Low; M=Medium, H=High SO_2 treatment

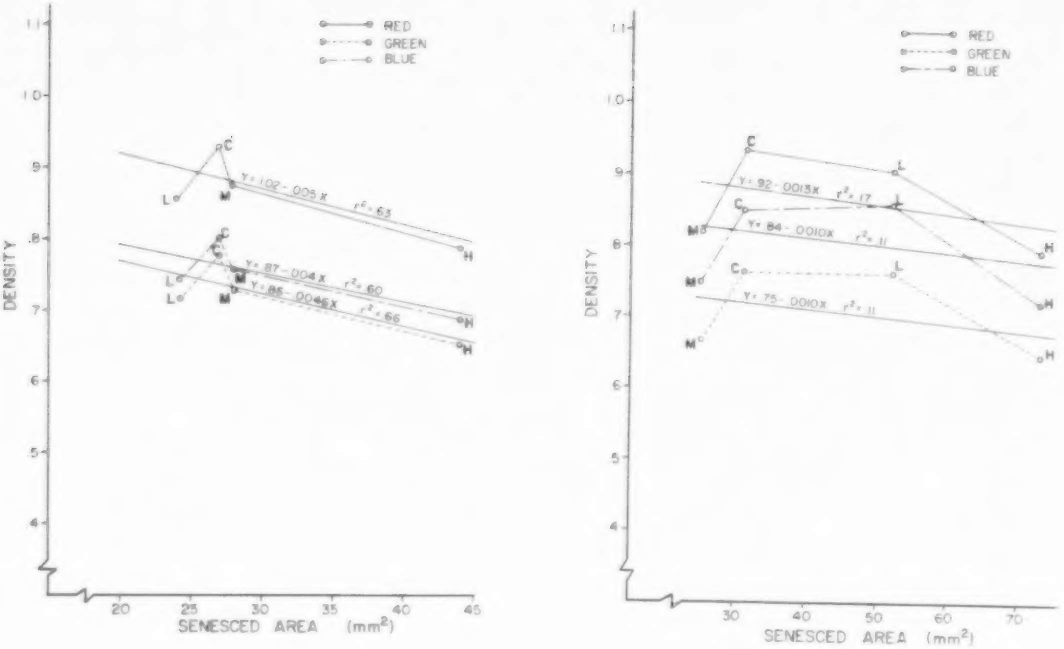


Figure 24.5. Density of May color photography over ZAPS I (left) and ZAPS II (right) related to senesced leaf area of western wheatgrass.

[1] C=Control; L=Low; M=Medium; H=High SO_2 treatment

Vegetation Mapping

Vegetation maps of the ZAPS plots are Figures 24.6. through 24.13. The index to map units is given in Table 24.1.

General Discussion

All the field elements of the CFPP Project are affected by site factors which have not been quantified. Perhaps the most serious of these is the notable increase in plant vigor which has occurred due to grazing deferment of the study sites. This introduces a potentially serious confounding factor with any low-level pollution effects.

We studied one of the sites (Kluver West) to see how much photo density differed between the inside and outside the exclosure (Figure 24.14.)

In all three colors there is a highly significantly greater density inside. Ground observations show a much more vigorous vegetational cover inside. If grazing deferment is coupled with low chronic pollution levels, it is very likely that any pollution effects would be masked by the deferment.

TABLE 24.1. INDEX TO MAP UNITS

SYMBOL	COMMUNITY
Sedges and Rushes	
AA	*Carex pennsylvanica/Agropyron smithii*
AB	*C. pennsylvanica/A. smithii/Stipa viridula*
AC	*C. pennsylvanica/A. smithii/Tragopogon dubius*
AD	*C. pennsylvanica/Artemisia ludoviciana*
AE	*C. pennsylvanica/Festuca idahoensis*
AF	*Juncus interior*
AG	*Carex* species
Grasses	
AH	*Agropyron cristatum*
AI	*A. smithii*
AJ	*A. smithii/Koeleria cristata/Poa sandbergii*
AK	*A. smithii/K. cristata*
AL	*A. smithii/S. viridula/P. sandbergii/K. cristata*
AM	*Agropyron spicatum/A. smithii*
AN	*Aristida longiseta*
AO	*Bouteloua gracilis*
AP	*B. gracilis/K. cristata*
AQ	*Bromus japonicus*
AR	*B. japonicus/A. smithii/Poa*
AS	*B. japonicus/Buchloe dactyloides*
AT	*B. japonicus/Cirsium undulatum*
AU	*B. japonicus/Stipa comata*
AV	*B. japonicus/Taraxacum officinale/A. smithii*
AW	*Calamovilfa longifolia*
AX	*C. longifolia/*mixed forbs
AY	*C. longifolia/Schizachyrium scoparium/A. smithii*
AZ	*F. idahoensis*
Aa	*Hordeum jubatum*
Ab	Mixed grasses
Ac	*Poa pratensis*
Ad	*P. pratensis/Achillea millefolium/A. smithii*
Ae	*P. pratensis/A. smithii*
Af	*P. pratensis/A. smithii/K. cristata*
Ag	*P. pratensis/A. spicatum*
Ah	*P. pratensis/C. pennsylvanica*
Ai	*P. pratensis/C. pennsylvanica/A. smithii*
Aj	*P. pratensis/S. viridula*
Ak	*P. pratensis/S. viridula/A. millefolium/A. smithii*
Al	*P. pratensis/S. viridula A. smithii*
Am	*P. pratensis/Vicia americana*
An	*S. comata/A. smithii/T. dubius*
Ao	*S. viridula*

TABLE 24.1. (continued)

SYMBOL	COMMUNITY
Grasses (continued)	
Ap	*S. viridula/A. smithii*
Aq	*S. viridula/A. smithii/Muhlenbergia cuspidata*
Ar	*S. viridula/Artemisia frigida/B. japonicus*
As	*S. viridula/V. americana*
Forbs	
BA	*A. millefolium*
BB	*A. millefolium/A. smithii*
BC	*A. millefolium/T. dubius*
BD	*Antennaria* species
Be	*Artemisia ludoviciana*
BE	*A. ludoviciana/A. smithii/K. cristata*
BF	*Bahia oppositifolia*
BG	*Cirsium undulatum*
BH	*Glycyrrhiza lepidota*
BI	*Grindelia squarrosa*
BJ	*G. squarrosa/Psoralea argophylla/P. pratensis/ A. ludoviciana*
BK	*Heterotheca villosa*
BL	*Hyoscyamus niger*
BM	*Lupinus sericeus*
BN	*Melilotus officinalis*
BO	*Medicago sativa*
BP	*M. sativa/A. cristatum*
BQ	*Monarda fistulosa*
BR	*Orthocarpus luteus/A. smithii/V. americana*
BS	*O. luteus/C. pennsylvanica/A. longiseta/F. idahoensis*
BT	*O. luteus/P. pratensis*
BU	*Oxytropis* species
BV	*P. argophylla/A. smithii/C. pennsylvanica*
BW	*P. argophylla/A. smithii/C. pennsylvanica/ M. cuspidata*
BX	*P. argophylla/A. smithii/S. comata*
BY	*P. argophylla/C. pennsylvanica*
BZ	*P. argophylla/Lupinus sericeus*
Ba	*P. argophylla/P. pratensis*
Bb	*P. argophylla/S. viridula*
Bc	*Solidago missouriensis*
Bd	*Solidago* species
Bf	*T. officinale/A. smithii/V. americana*
Bg	*T. officinale/P. pratensis*
Bh	*T. officinale/Sphaeralcea coccinea/A. smithii*

TABLE 24.1. (continued

SYMBOL	COMMUNITY
Forbs (continued)	
Bi	*T. dubius/A. smithii*
Bj	*T. dubius/A. smithii/A. frigida*
Shrubs and Half-Shrubs	
CA	*Artemisia cana*
CB	*A. cana*/mixed grasses
CC	*Artemisia dracunculus*
CD	*A. frigida*
CE	*A. frigida/A. smithii*
CF	*A. frigida/S. comata/A. smithii*
CG	*A. ludoviciana/C. pennsylvanica/Symphoricarpos/ S. viridula*
CH	*A. ludoviciana/Artemisia tridentata/A. smithii/ S. viridula*
CI	*A. tridentata*
CJ	*A. tridentata*/mixed grasses
CK	*A. tridentata A. cana*/mixed grasses
CL	*Atriplex nuttallii*
CM	*Ceratoides lanata*
CN	*C. lanata*/mixed grasses
CO	*Juniperus horizontalis*
CP	*Rhus trilobata*
CQ	*R. trilobata/Yucca glauca/A. tridentata*
CR	*R. trilobata/Lupinus sericeus/Schizacyrium scoparium*
CS	*Rosa woodsii*
CT	*Symphoricarpos occidentalis*
CU	*Xanthocephalum sarothrae*
CV	*X. sarothrae/A. smithii/P. sandbergii/A. millefolium*
Trees	
DA	*Salix* species
Non-Vegetation Units	
✱	Anthills, Bare Ground, or Disturbed Sites
×	Burn
▲	Grasshopper Cages
■	Photoplots
⬡	SO_2 Collection Plates

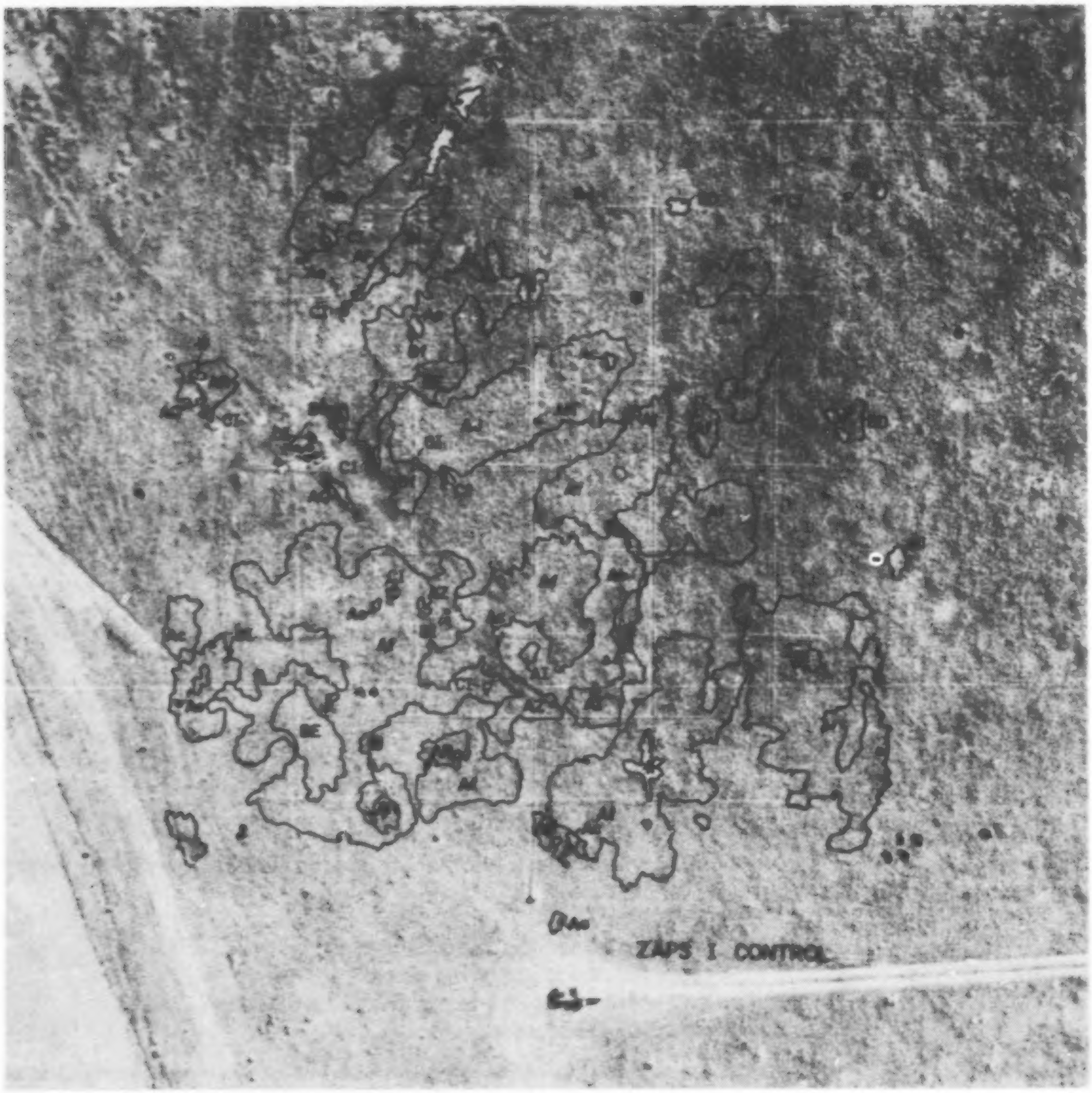

ZAPS I CONTROL

Figure 24.6. Vegetation map, ZAPS I control. Scale = 1:800
(For key to map units see Table 24.1. p. 911-913)

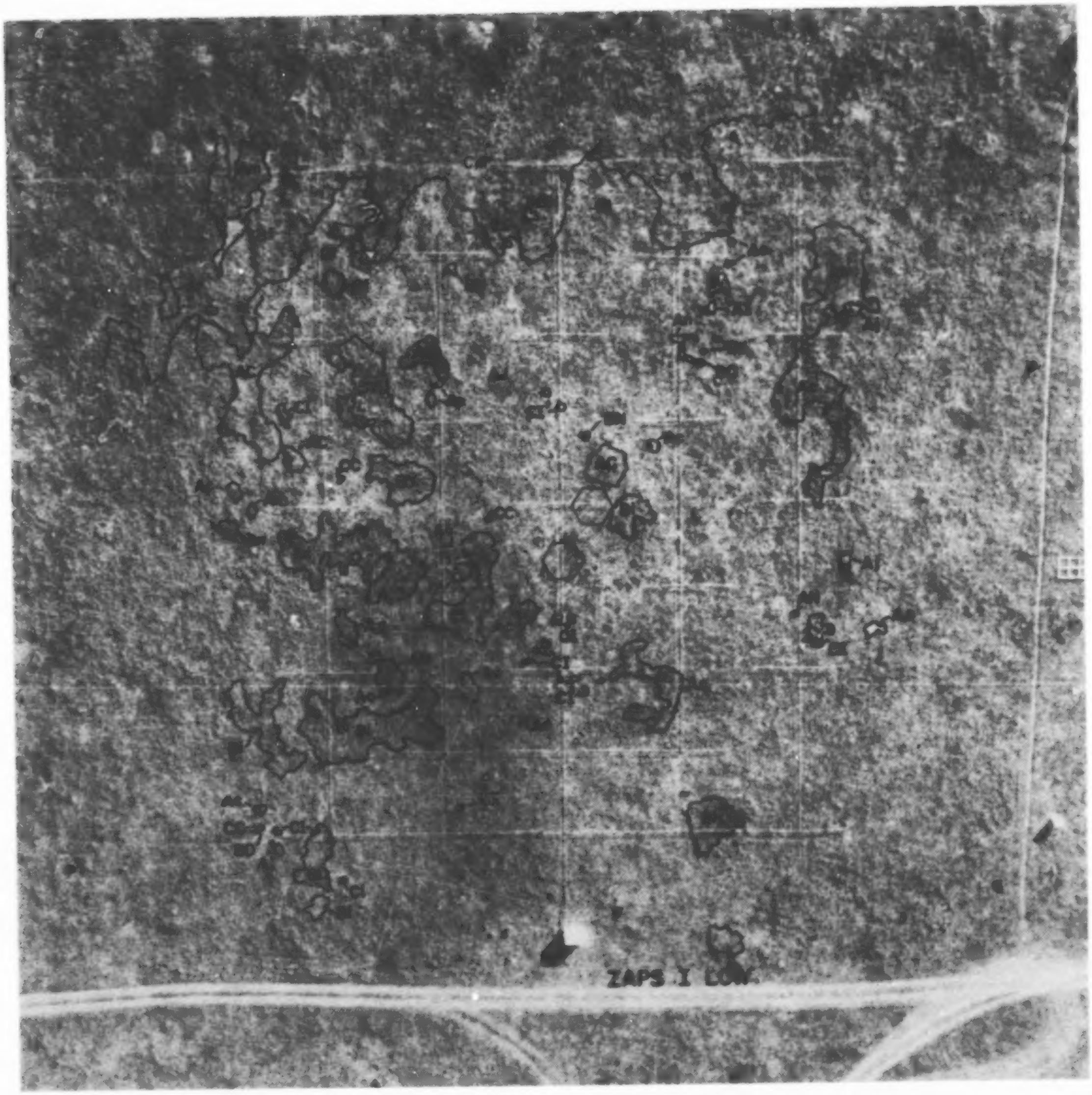


Figure 24.7. Vegetation map, ZAPS I low. Scale = 1:800.
(For key to map units see Table 24.1. p. 911-913)

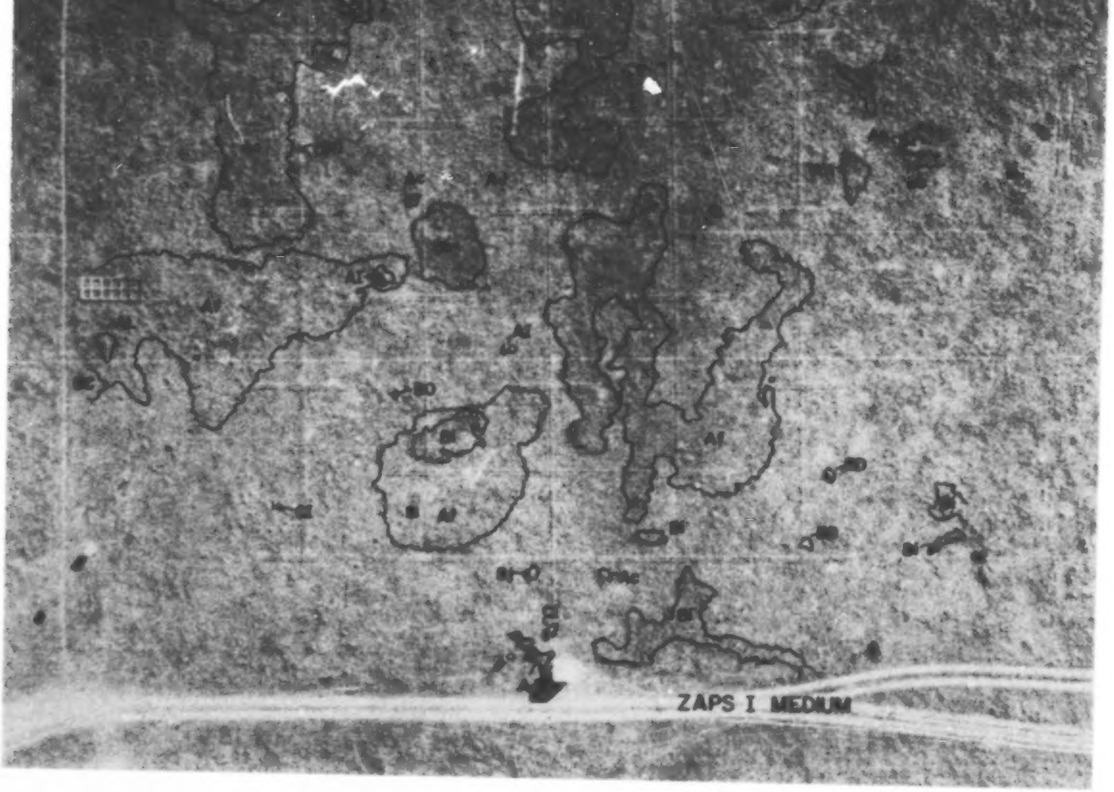

Af
Af
Af
Af
Af
Af
ZAPS I MEDIUM

Figure 24.8. Vegetation map, ZAPS I medium. Scale = 1:800.
(For key to map units see Table 24.1. p. 911-913)

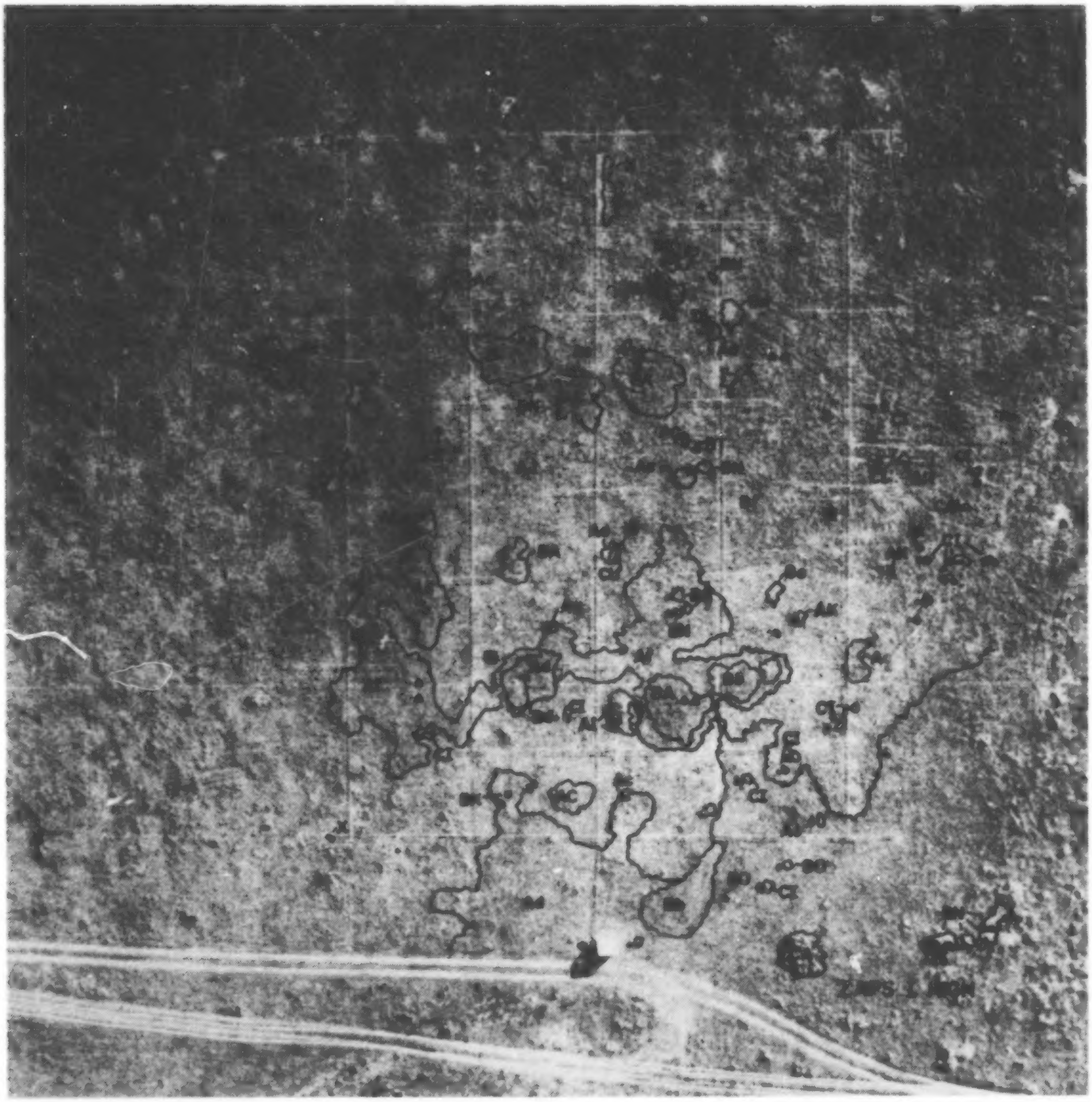


Figure 24.9. Vegetation map, ZAPS I high. Scale = 1:800.
(For key to map units see Table 24.1. p. 911-913)

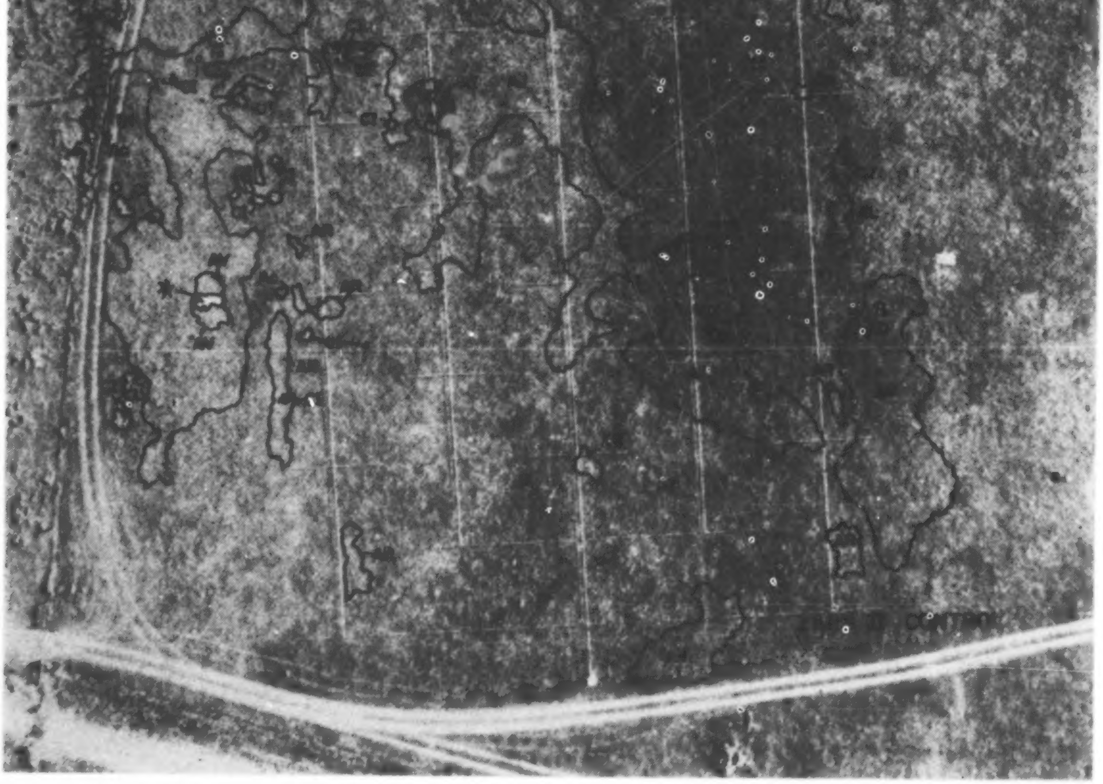

ZAPS II CONTROL

Figure 24.10. Vegetation map, ZAPS II control. Scale = 1:800. (For a key to map units see Table 24.1. p. 911-913)

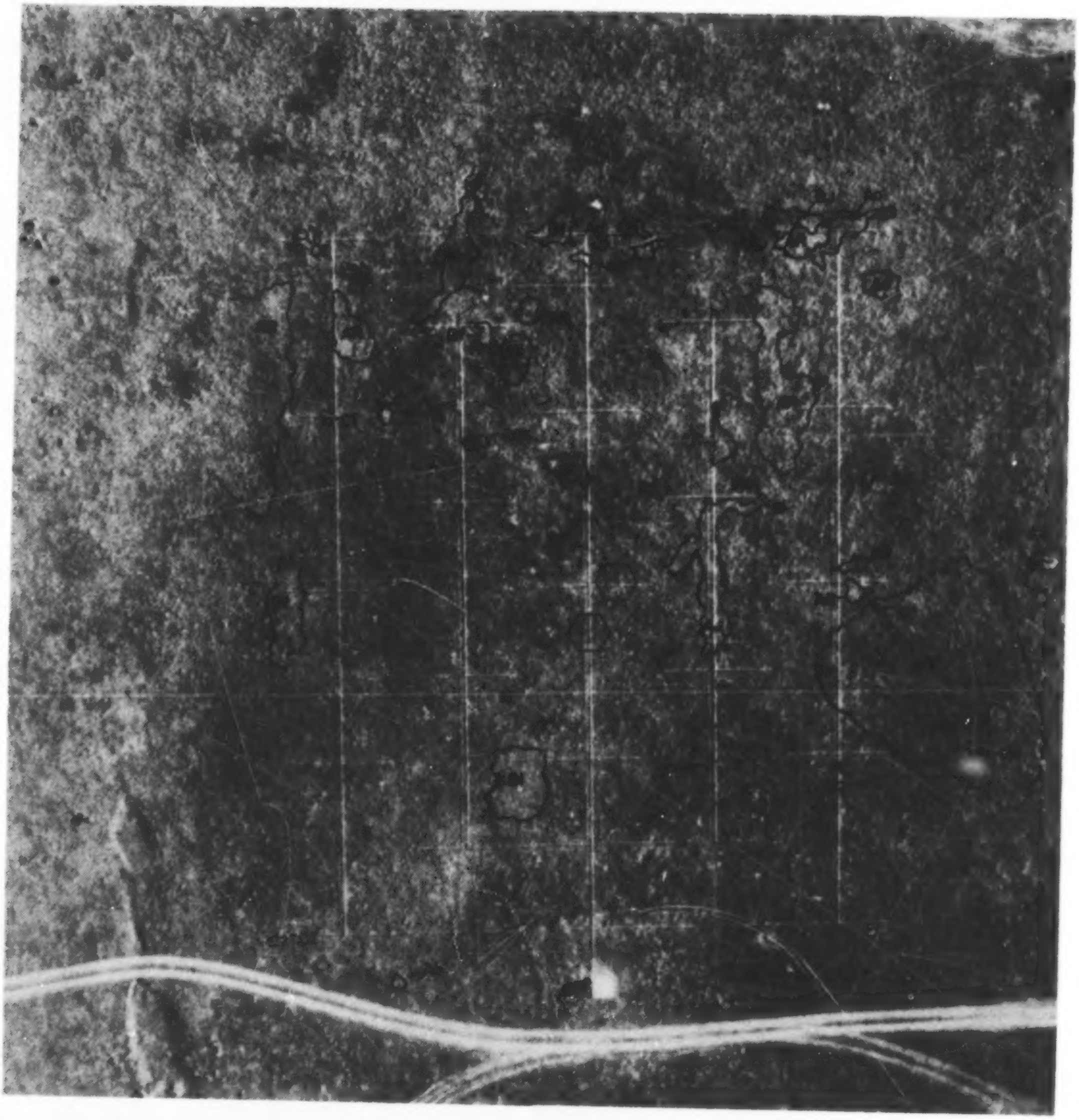


Figure 24.11. Vegetation map, ZAPS II low. Scale = 1:800.
(For a key to map units see Table 24.1. p. 911-913)

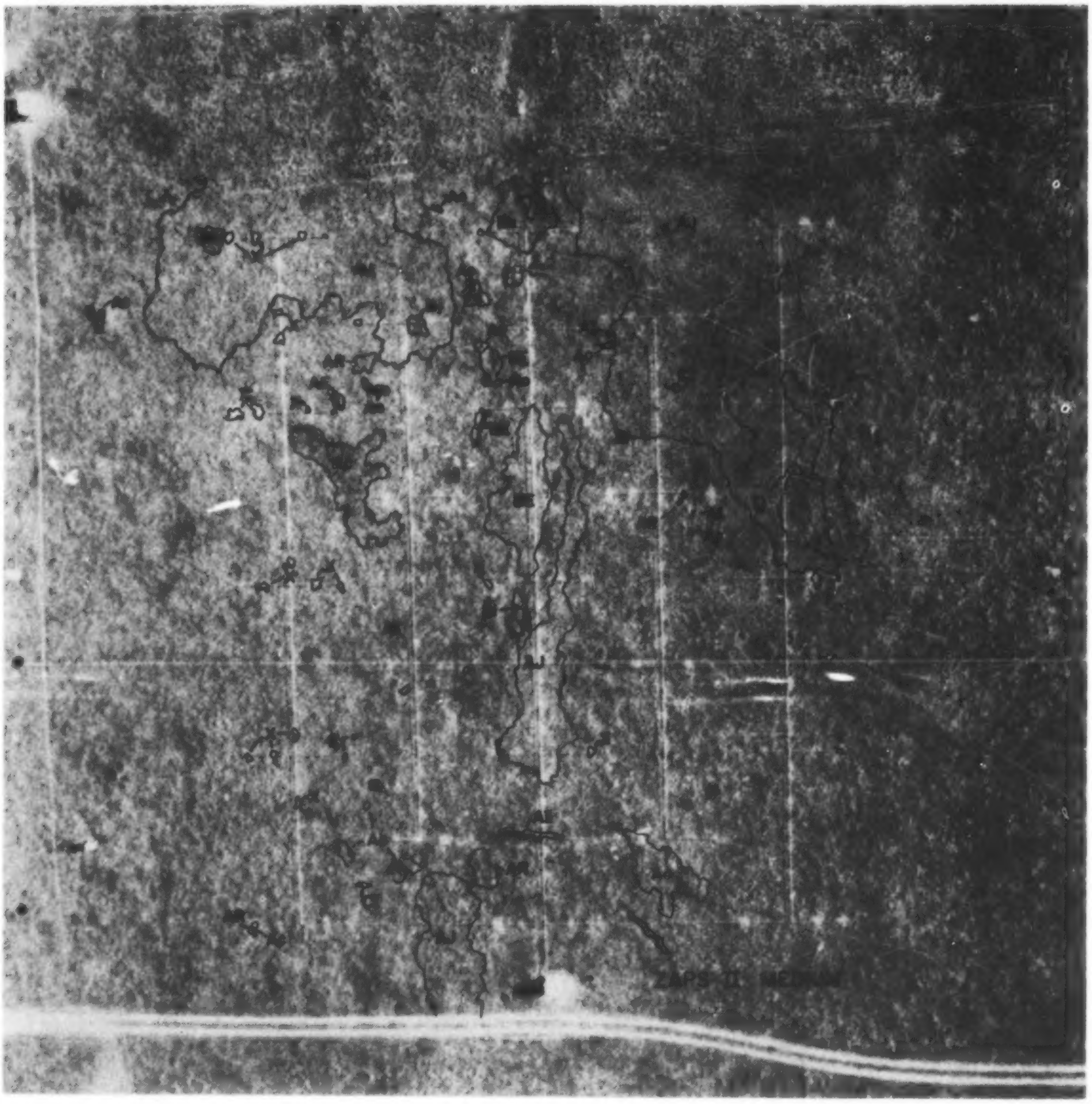

ZAPS II MEDIUM

Figure 24.12. Vegetation map, ZAPS II medium. Scale = 1:800.
(For a key to map units see Table 24.1. p. 911-913)

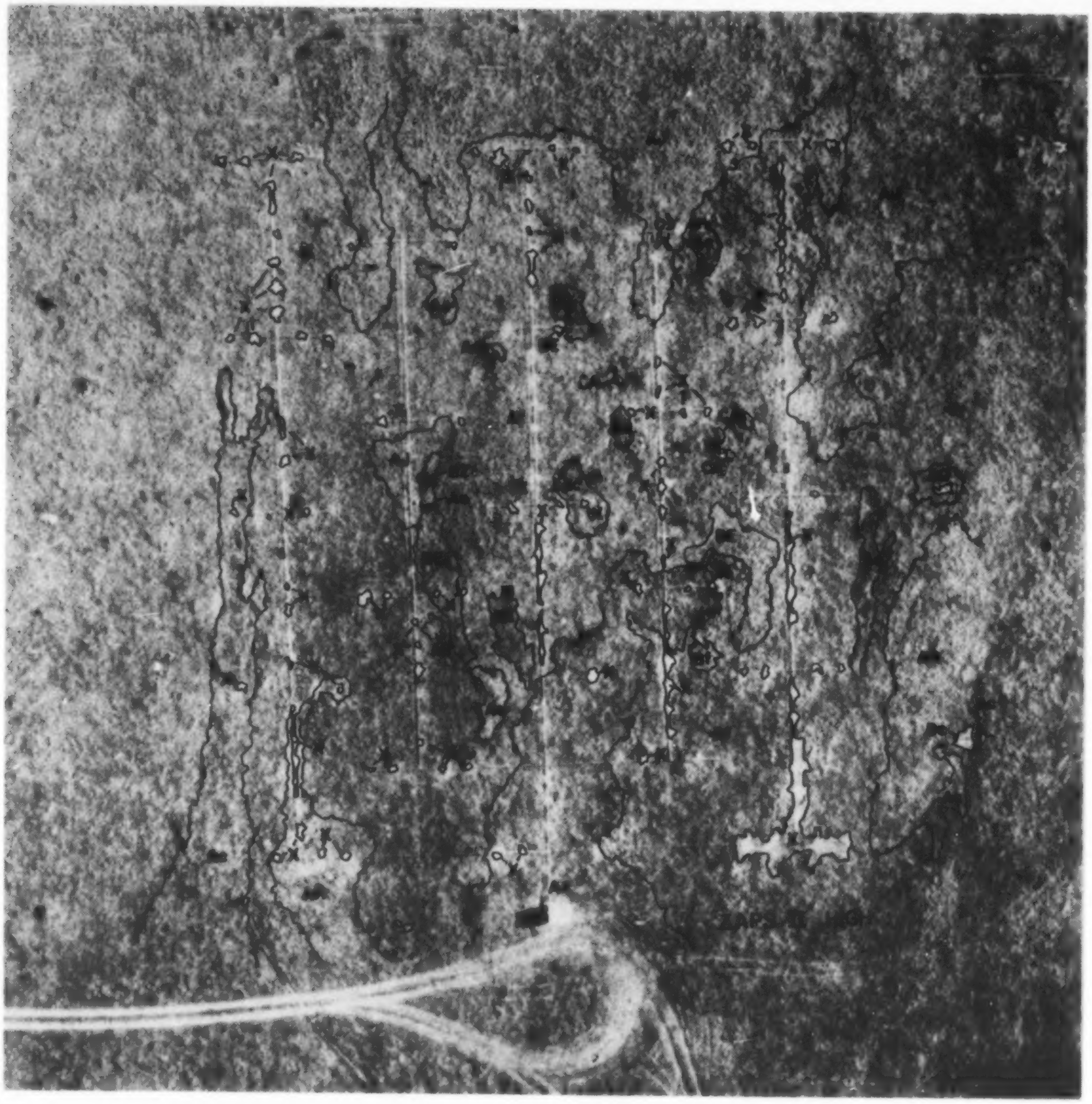


Figure 24.13. Vegetation map, ZAPS II high. Scale = 1:800.
(For a key to map units see Table 24.1. p. 911-913)

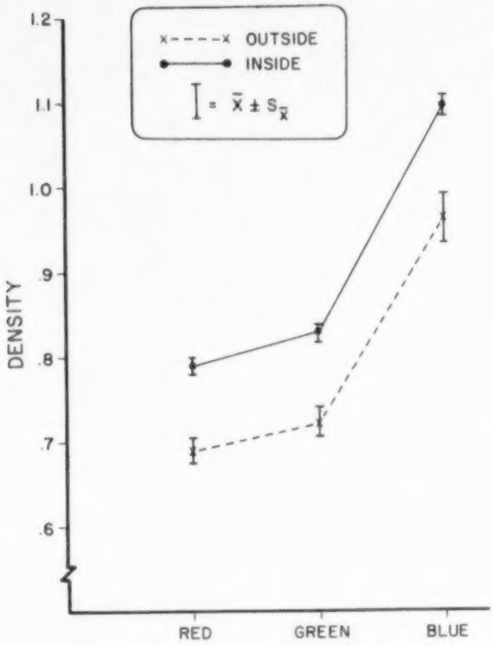


Figure 24.14. Optical density comparison between inside and outside the Kluver West exclosure.

In the absence of comparable grazed plots, the exclosure data are highly questionable. This problem is substantially diminished with the McRae Knoll relict sites along Rosebud Creek. They can be considered baseline examples of several vegetational types in the general area of Colstrip, so partially serve as control sites for the exclosure.

Another factor which can affect data is grasshopper infestations. These occur periodically, and can be severe enough to invalidate sampling some years. In 1977 the Kluver West site was very severely infested. Along with annual variations in climate, this emphasizes the importance of long term studies when dealing with grassland ecosystems.

CONCLUSIONS

An aerial photography system has been developed and is being used to monitor plant communities in southeastern Montana. Large scale growing season coverage with color infrared film records plant species and communities at high resolution. This photography has been used to map the study sites at McRae Knolls and ZAPS.

Visual observation, the Munsell Color System, image enhancement and densitometric analysis give successively more useful information from the imagery. Highly significant relationships have been observed between photographic density and theoretical SO_2 levels. In addition, the ZAPS I June photography shows close agreement between photo density and leaf senescence in western wheatgrass.

REFERENCES

Anderson, J.S. 1978. Large Scale Aerial Photography over Native Range Transects. M.S. Thesis. Montana State University, Bozeman, 96 p.

Anderson, J.S., J.E. Taylor, and W.C. Leininger. 1977. Large Scale Aerial Photography of a Native Range Transect. Abstr. Ann. Mtg. Soc. Range Manage., Portland, Oregon, p. 7.

Munsell Corporation, 1976. Munsell Book of Color. Munsell Color, Macbeth Div. of Kolmorgen, Inc., Baltimore, Md.

Piech, K.R. et al. 1974. Method for Extracting Photometric Information from Aerial Photographic Imagery. U.S. Patent #3,849,006.

Schott, J.R., D.W. Gaucher, and J.E. Walker. ND. Aerial Photographic Technique for Measuring Vegetation Stress from Sulfur Dioxide. Calspan Corp. Rep. YB-5967-M-1.

APPENDIX 24 . 1.

LARGE SCALE AERIAL PHOTOGRAPHY FLOWN IN 1977 OVER EPA STUDY SITES

DATE	SITE	SCALE	EMULSION TYPE	FILTER
22 May	ZAPS I & II	1:15,000	2443(70mm CIR)	Wratten 15
		1:15,000	2448(70mm color)	Haze
		1:3700	2443	Wratten 15
	McRae Knolls	1:3700	2443	Wratten 15
	Kluver West	1:3700	2443	Wratten 15
	Kluver North	1:3700	2443	Wratten 15
22 June	ZAPS I & II	1:10,000	2443	Wratten 15
		1:10,000	120-VPS	Haze
		1:3700	120-VPS	
		1:3700	2443	Wratten 15
	McRae Knolls	1:3700	2443	Wratten 15
		1:3700	2448	
	Hay Coulee	1:3700	2448	
		1:3700	2443	Wratten 15
	Kluver West	1:3700	2443	Wratten 15
		1:3700	2448	
	Kluver North	1:3700	2448	
		1:3700	2443	Wratten 15
	Kluver East	1:3700	2443	Wratten 15
		1:3700	2448	
27 July	ZAPS I & II	1:20,000	2443	Wratten 15
		1:20,000	2448	Haze
		1:10,000	2448	Haze
		1:10,000	2443	Wratten 15
9 August	McRae Knolls	1:2700	120-VPS	
		1:1500	120-VPS	
	Hay Coulee	1:1500	120-VPS	
		1:900	120-VPS	
	Kluver West	1:2700	120-VPS	
		1:1500	120-VPS	
	Kluver North	1:2700	120-VPS	
		1:1500	120-VPS	
	Kluver East	1:1500	120-VPS	
		1:900	120-VPS	
31 August	ZAPS I & II	1:12,000	120-VPS	
		1:3700	120-VPS	
		1:3700	2443	Wratten 15
	McRae Knolls	1:3700	2443	Wratten 15
		1:3700	120-VPS	

APPENDIX 24.2.

SMALL SCALE PHOTOGRAPHY OF EPA STUDY SITES IN SOUTHEASTERN MONTANA

DATE	COVERAGE	SCALE	EMULSION TYPE	ACQUIRING AGENCY
28 August 1973	ZAPS I & II Sites Custer National Forest, Ashland/Ft.Howes Districts	1:36,000	Color Infrared (CIR)	U.S. Forest Service, U.S. Department of Agriculture
28 June 1974	Colstrip Mines and Surrounding Region (ZAP Site Excluded)	1:80,000	CIR	U.S. Bureau of Land Management, U.S. Department of Interior
14 May 1975	Colstrip Mine Area North and Eastward to Rosebud Creek	1:110,000		National Aeronautics and Space Administration (NASA)
23 June 1975	Colstrip Mine Area North and Eastward to Rosebud Creek	1:55,000	CIR	NASA
16 October 1975	Colstrip Mines and Environs to Rosebud Creek; ZAPS I & II	1:55,000	SO 397, Color 2443, CIR	NASA
22 July 1976	ZAPS Sites I & II	1:24,000	2443, CIR 2448, Color	U.S. Environmental Protection Agency/EPIC

SECTION 25

METHODOLOGY DEVELOPMENT FOR SITING POWER PLANTS

J. L. Dodd, W. K. Lauenroth and W. J. Parton

Within the past year we have structured a research and work plan for the development of a methodology that can be applied to the northern Great Plains or other similar regions for the purpose of classifying all parts of the region according to their anticipated reduction in ecological value resulting from air pollution impact from coal-fired power plants. The work will be restricted to air pollution impacts on the major terrestrial ecosystem types of the area (native and crop systems). Since the ecosystem types are important to society in more than one way, an attempt will be made to rate their sensitivity to air pollution impact according to several criteria. The major value systems to be considered are wildlife habitat value, agricultural productivity, and recreational-aesthetic values.

We are suggesting a two-phase system for evaluating regions for *candidate areas* for power plant sites. The system will consist of a low resolution screening system capable of evaluating all possible sites within a region and a high resolution system that would be utilized to anticipate specific impacts on a small number of sites under serious consideration. We will attempt to develop a low resolution system (LRES) that

1. Can be applied by decision makers with a limited amount of technical assistance by resource specialists.
2. Is transferrable from one region to another without great changes in the basic framework of the methodology.
3. Requires a data base that is comparatively easy to secure.
4. Has sufficient flexibility to allow decision makers to easily specify the relative importance of the various value systems.
5. Can be improved in a straightforward manner by updating with new information on air pollution effects as data becomes available from scientific research.

The second system will be a high resolution system (HRES) designed to evaluate a single or a very small set of potential sites. The high resolution ecological evaluation system will consist of a collection of simulation and regression models that are designed to simulate effects of air pollution on each cover type that occurs in the region. Basic frameworks for these models will be existing models where possible. The existing models will be modified to respond to air pollution stress and to show influence of air pollution stress on the various value systems.

This effort will differ from the low resolution analysis in many ways. It will require much more specific information about the cover types, soils, and abiotic characteristics of each cover type and will require heavy involvement by modeling and resource specialists. Application of the high resolution ecological evaluation system will probably require one field season of data collection to secure information for initializing and tuning the models to an area chosen as a possible power plant site.

Simulation models for a variety of ecosystems and agricultural crops have been developed and are just starting to be utilized in environmental impact analysis. The natural ecosystem models developed by the different biomes (grassland, desert coniferous forest, and deciduous forest) in the U.S. International Biological Program have been used to assess the impact of SST development on natural systems (Cooper *et al.* 1974) while the ELM grassland model (Innis 1978) has been used to simulate the impact of weather modification (Parton and Smith 1974). A discussion of the problem associated with utilizing these models in environmental analyses is presented by the Holcomb Research Institute (1976) and Parton and Wright (1977).

The data base for the two systems will differ considerably. In the first case (low resolution system) the data base will be based on available interpretations of remote sensing imagery. In the second case (high resolution system) the data base will require specific biological and ecological data collected from each of the potential sites after their preliminary selection.

The low and high resolution ecological evaluation systems will ultimately interface with the overall power plant siting process in one of several possible ways. Since a standard structure for the total power plant siting process does not exist, we present two alternatives below and indicate how our proposed ecological evaluation systems would fit into each.

In the first case, separate low resolution analyses for ecological, socio-economic, and engineering considerations would be conducted in a parallel fashion (Figure 25.1). All possible sites would be evaluated by each system and the better sites for power plant siting would be so indicated. The decision-making body would then select only those sites rated as the better sites in all three low resolution evaluations. This would substantially reduce the number of sites that need to be evaluated by much more costly and time-consuming high resolution analyses.

A single candidate site or group of better sites would then be selected by the decision makers and be subjected to high resolution evaluation (ecological, socio-economic, and engineering, in parallel). Based upon results of the high resolution analyses (i.e., projections of consequences of

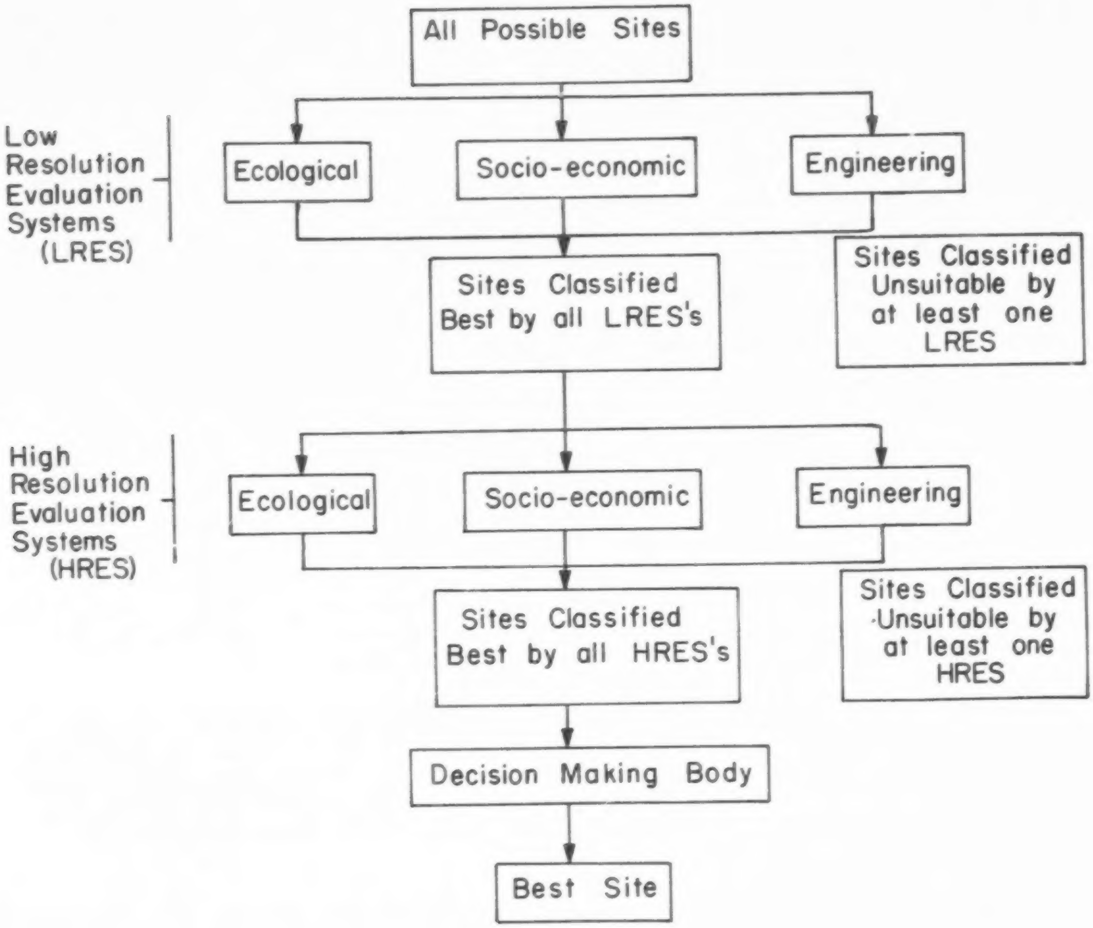

All Possible Sites
Low Resolution Evaluation Systems (LRES)
Ecological
Socio-economic
Engineering
Sites Classified Best by all LRES's
Sites Classified Unsuitable by at least one LRES
High Resolution Evaluation Systems (HRES)
Ecological
Socio-economic
Engineering
Sites Classified Best by all HRES's
Sites Classified Unsuitable by at least one HRES
Decision Making Body
Best Site

Figure 25.1. Power plant site selection process--Parallel application of evaluation systems.

siting a power plant at a particular locale), the decision-making body could then select the best site or reject all sites.

An alternative power plant siting process would consist of the same elements as in the process discussed previously but would differ in having the three low resolution and high resolution evaluations applied sequentially (Figure 25.2). In this configuration of the process one low resolution evaluation system would be applied to all possible sites; the second low resolution evaluation would then be applied to only the sites deemed better by the first LRES and so forth. The three high resolution evaluation systems would be applied in the same manner. The final step of the process would be carried out by the decision-making body in the same manner as for the parallel application of the evaluation systems (Figure 25.1).

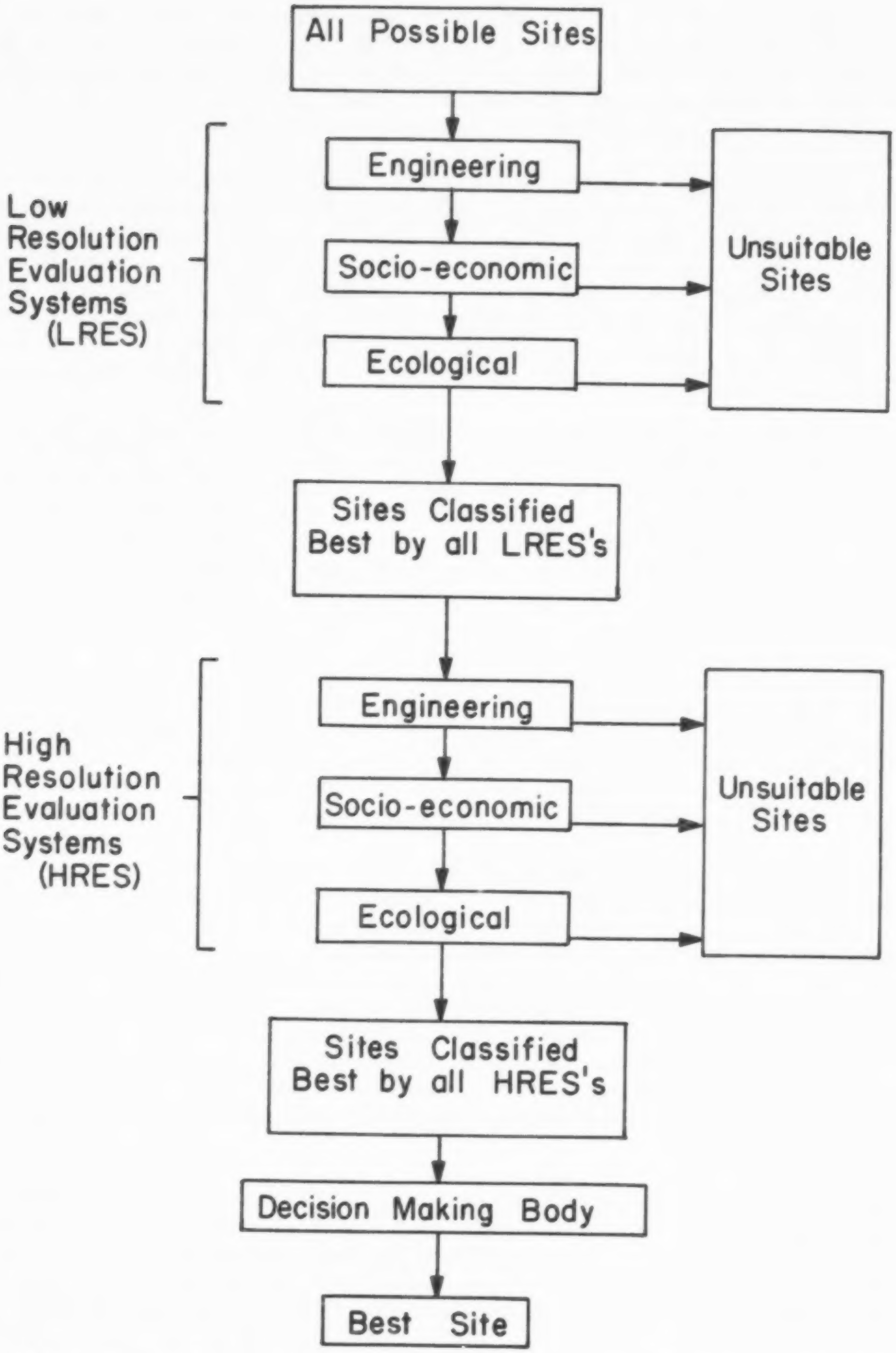

All Possible Sites
Low Resolution Evaluation Systems (LRES)
Engineering
Socio-economic
Ecological
Unsuitable Sites
Sites Classified Best by all LRES's
High Resolution Evaluation Systems (HRES)
Engineering
Socio-economic
Ecological
Unsuitable Sites
Sites Classified Best by all HRES's
Decision Making Body
Best Site

Figure 25.2. Power plant site selection processes--Sequential application of evaluation systems.

A number of advantages and disadvantages exist for both forms of the siting processes discussed. One problem that may be common to both, as illustrated in Figures 25.1 and 25.2, is that it may well be necessary to have middle level resolution evaluation phases in order to reduce the number of potential sites that have to be subjected to the HRES's.

An obvious advantage of the sequential applications structure is that the second and third evaluation systems would have to consider fewer possible sites than the first evaluation (at any level of resolution). This might effect a reduction in cost of the power plant siting process itself. A possible disadvantage of the sequential system is that it would not allow the user to see sites that are classified best by two out of three of the evaluation systems (essentially second best sites) except when the sites were classified as acceptable by the first two evaluations of the sequence.

At the present time a variety of methodologies are used in the power plant siting procedure. A common feature of most of these techniques is that they start by screening large areas and then narrow down the search to a smaller number of potentially suitable sites. The most widely used technique for initial screening is the use of map overlays. Maps of the region of interest are made on transparent film, with each map shaded to represent the effect of a particular site consideration (darker shading indicates lower site suitability). Some of the site considerations include: site development criteria, such as water supply, land availability, and seismology; environmental criteria, ranging from water quality to the effect of air pollution on the environment; and socio-economic criteria. A visual screening of the maps overlaid upon each other will show the area that appears lightest and thus is the most acceptable for further site analysis. The siting procedure starts by determining a number of *candidate areas* within the overall region and then narrowing down to a small number of candidate sites.

This procedure can be quantified by assigning values for each site criterion on each given point in the region and then using the computer to generate maps for each criterion or computer maps which combine any subset of the different criteria and thus show a combined suitability for each given unit. A variety of different groups [Argonne National Laboratory (Frigeria *et al.* 1975), Dames and Moore (Fischer and Ahmed 1974), and EDAW, Inc. (Bishop 1972)] have been successful at quantifying the power plant siting procedure utilizing computer mapping, data analysis, and computer simulation models. A thorough comparison of the methodologies used in power plant siting is presented in a report by the MITRE Corporation (Graf-Webster *et al.* 1974).

Details of our research plan are contained in a research proposal that has been submitted to the Environmental Protection Agency. Pending approval of the proposal, work will be initiated in late summer of 1978. We anticipate completion of the low resolution ecological evaluation system by the end of 1979 and completion of the high resolution system by the end of 1980.

REFERENCES

Bishop, A. B. 1972. Approach to Evaluating Environmental Soil and Economic Factors. Water Resour. Bull., 8(4):724-734.

Cooper, C. F., T. J. Blasing, H. C. Fritts, Oak Ridge Systems Ecology Group, F. M. Smith, W. J. Parton, G. F. Schreuder, P. Sollins, J. Zich, and W. Stoner. 1974. Simulation Models of the Effects of Climatic Change on Natural Ecosystems. In: Proceedings of the Third Conference on the Climatic Impact Assessment Program. Transportation Systems Center, U.S. Dep. Transportation, Cambridge, Mass. pp. 550-562.

Fischer, J. A., and R. Ahmed. 1974. A Systematic Approach to Evaluate Sites for Nuclear Power Plants. Presented at the Conference on Nuclear Power Plant Siting, American Nuclear Society, 26-28 August, Portland, Oregon.

Frigeria, N. A., L. J. Habegger, R. F. King, L. J. Hoover, N. A. Clark, and J. M. Cabian. 1975. Site: A Methodology for Assessment of Energy Facility Siting Patterns. ANL/AA-2 Argonne National Laboratory, Argonne, Ill. pp. 1-116.

Graf-Webster, E., S. Haus, S. Labore, J. Pfeffer, and J. Watson. 1974. Resource and Land Investigations (RALI) Program: Methodologies for Environmental Analysis--Vol. III. Power Plant Siting. Final Report. Contract 14-08-0001-14715, Report No. USGS-LI-75-005. U.S. Geological Survey, Reston, Va.

Holcomb Research Institute. 1976. Environmental Modelling and Decision Making. Praeger Publishers, New York.

Innis, G. S., ed. 1978. Grassland Simulation Model. Ecol. Studies, Vol. 26. Springer-Verlag, Inc., New York. 298 p.

Parton, W. J., and F. M. Smith. 1974. Exploring Some Possible Effects of Potential SST-Induced Weather Modification in a Shortgrass Prairie Ecosystem. In: Sixth Conference on Aerospace and Aeronautical Meteorology. American Meteorological Society, Boston, Mass. pp. 255-258.

Parton, W. J., and R. G. Wright. 1977. The Use of Models in the Analysis of Environmental Impact. In: New Directions in the Analysis of Ecological Systems, Part 1, G. S. Innis, ed. Simulation Councils, Proc. Ser. Vol. 5, No. 1. The Society for Computer Simulation (Simulation Councils, Inc.), La Jolla, Calif. pp. 83-92.